Cranfield University, Silsoe

Institute of BioScience and Technology

Postharvest Group

PhD Thesis

Academic Year 2002-2003

Anastasios I. Darras

Biology and management of freesia flower specking caused by *Botrytis cinerea*

Supervisors: Daryl C. Joyce & Leon A. Terry

This thesis is submitted in fulfilment of the requirement of the Degree of Doctor of Philosophy

Dedicated to Yannis, Despina and Constantinos

'Οποιος φτάνει το ιδανικό του, απ'αυτό το ίδιο το γεγονός
το ξεπερνά κιόλας

Φ. Νίτσε

ABSTRACT

There is no published research regarding postharvest infection of freesia flowers by *Botrytis cinerea*. Although, infection problems have concerned freesia growers and wholesalers in recent years. The overall objectives of this study were firstly to evaluate the factors affecting *B. cinerea* postharvest disease establishment and secondly to evaluate a range of novel potential treatments to reduce postharvest freesia infection. These treatment options include plant activators such as acibenzolar-S-methyl and methyl jasmonate and biotic (*Aureobasidium pullulans*) and abiotic (UV-C irradiation) biological/elicitors agents.

Research was undertaken in an attempt to explain the variation in *B. cinerea* incidence on cut freesia flowers as noted by the UK importer Zwetsloots & Sons Ltd. in 2000. Higher monthly rejections of freesia flower stems throughout 2000 due to *B. cinerea* infection were recorded during spring (April-May), early summer (June) and autumn (October). Comparatively higher proportions of rejected freesia stems were associated with glasshouse temperatures ranging from 13-17°C.

In the presence of *B. cinerea* inoculum on freesia petal surface, temperature was not a limiting factor for disease establishment. Incubation of artificially inoculated freesia flowers at 12°C resulted in overall higher disease severity and lesion numbers compared to flowers incubated at 5 or 20°C. In contrast, relative humidity was the most important factor for postharvest infection by *B. cinerea*.

Elicitor based strategies for IPM using the potent activator acibenzolar provided limited protection of freesia flowers against *B. cinerea* when applied postharvest. Acibenzolar significantly reduced disease severity, lesion numbers and lesion diameters compared to the untreated control when applied at 0.15 g A.I. L^{-1}.

Methyl jasmonate (MeJA) applied as gas, pulse and spray generally suppressed *B. cinerea* disease on cut freesia flowers. Disease severity, lesion numbers and lesion diameters of flowers gassed with 0.1 μL MeJA L^{-1} were reduced by 56, 43 and 37%, respectively compared to untreated control flowers. Gaseous MeJA treated freesia flowers at 0.1 μL L^{-1} increased PPO activity by 57% compared to untreated controls 24h after MeJA treatment. After 36h of incubation at 20°C, disease severity, lesion numbers

and lesion diameters of gaseous MeJA treated flowers were reduced by 68, 56 and 50%, respectively, compared to the untreated controls. However, PAL activity in MeJA treated freesia flowers did not decrease significantly over time compared to untreated control 12h post-inoculation and thereafter. These findings suggest that MeJA treatment might suppress the action of PAL in the phenylpropanoid pathway and consequently block SA production.

UV-C irradiation might be used in an integrated postharvest disease management program for freesia flowers. UV-C irradiation after artificial inoculation resulted in markedly reduced *B. cinerea* disease severity scores and lesion numbers. In detail, UV-C irradiation of cut freesia flowers with 0.5, 1, 2.5 and 5 kJ m^{-2} reduced disease severity by up to 44, 70, 74 and 59% and lesion numbers by up to 37, 62, 68 and 60%, respectively. UV-C irradiation at 1 kJ m^{-2} before artificial inoculation slightly reduced disease severity and lesion numbers possibly by inducing defence responses. However, the limited disease suppression suggested that apparently *B. cinerea* could overcome the UV-C induced effect.

The effect of preharvest treatments on freesia crops with acibenzolar was investigated in glasshouse trials in view to suppress postharvest *B. cinerea* infection via SAR induction. Acibenzolar was effective in selected treatments and conditions. Disease pressure varied over the 3 years and over varieties tested. However, it was unclear whether acibenzolar induced systemic and/or local defence responses. The latter was supported by biochemical investigations in 2001 which suggested that acibenzolar did not induce PAL activity.

In contrast, preharvest MeJA treatment resulted in markedly systemic protection of treated flowers compared to untreated ones. MeJA efficacy was dependent on variety and on postharvest incubation temperatures. Disease severity, lesion numbers and lesion diameters on MeJA treated freesia var. 'Dukaat' flowers incubated at 20°C were reduced by 56, 61, and 49% compared to controls, respectively. Also, disease severity, lesion numbers and lesion diameters on MeJA treated 'Cote d'Azur' flowers incubated at 20°C were reduced by 36, 26, and 49% compared to controls, respectively.

ACKNOWLEDGEMENTS

I sincerely thank my supervisors, Professor Daryl Joyce and Dr Leon Terry for their guidance throughout the thesis. Their advice, encouragement, and persistence ultimately resulted in the completion of this research. I also sincerely thank Dr Irene Vloutoglou for the critical review of the thesis drafts.

Zwetsloots & Sons Ltd. (UK) are gratefully acknowledged for their financial support and enthusiasm for this project. I would like to personally thank those who helped me over this research, Mr David Zwetsloot, Miss Nicky Little, Mr Robert Earl, Mr Keith Richardson and Mr Mark Budgen. Special thanks are given to Mr Scott Zwetsloot for providing resources and Zwetsloots' glasshouse plantation for my preharvest trials.

I would like to personally thank the members of the thesis committee board Professor Naresh Magan, Professor Phill Warner and Miss Nicky Little. Thanks are bestowed to Mr Allen Hilton for his technical assistance, Mr Charles Marshall and Mr Ian Crawford for their advices on statistical analyses and Mr Paul Dauny; all made my life easier.

I thank the staff and students of Postharvest Group and those who shared after work moments of relaxation, especially Christos Dimitriadis, Nektarios Pompodakis, and Pericles Toukiloglou. Special thanks to my girlfriend Vasso who inspired and kept me motivated during this period.

TABLE OF CONTENTS

LIST OF PLATES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
Adomet	S-adenosyl-methionine
Ag^+	Silver
$AgNO_3$	Silver nitrate
$Al_2(SO_4)$	Aluminium sulfate
AI	Active ingredient
AIP	2-aminoindan-2-phosphonic acid
ANOVA	Analysis of variance
AOA	α-aminooxy acetic acid
AOPP	α-aminooxy-β-phenylpropionic acid
atm	Atmosphere
avr	Avirulent
BA	Benzoic acid
ca.	approximately
Ca^{2+}	Calcium
CA	Controlled atmosphere
CAD	Cinnamyl-alcohol dehydrogenase
CHS	Chalcone synthase
4CL	4-coumarate: CoA-ligase
cfu	Colony forming units
CK	Choline kinase
Cl^-	Chloride
cm	Centimetre
CMV-Y	Cucumber mosaic virus
CO_2	Carbon dioxide
coi1	JA-insensitive
CRB	Completely randomized block
cv.	Cultivar

°C	Degrees celcius
d	Day
e.g.	For example
ein2	Ethylene insensitive
ERH	Equilibrium relative humidity
et al.	And others
etr1	Ethylene resistant 1
fad3	Fatty-acid deficient 3
FW	Fresh weight
g	Gram
GA	Gibberellic acid
h	Hour
H^+	Hydrogen
H_2O_2	Hydrogen hyperoxide
8-HQC	8-hydroxyquinoline citrate
8-HQS	8-hydroxyquinoline sulfate
HR	Hypersensitive reaction
IAA	Indole acetic acid
i.e.	That is
IPM	Integrated pest management
IRA	Infra-red absorbing
ISR	Induced systemic resistance
JA	Jasmonic acid
jar1	JA resistant 1
K^+	Potassium
kg	Kilogram
L	Litre
LAR	Local acquired resistance
LOX	Lipoxygenase
1-MCP	1-methylcyclopropane
m	Metre

M	Molar
mL	Mililitre (10^{-3} L)
µL	Mictolitre (10^{-6} L)
µm	Micrometre (10^{-6} m)
µM	Micromole (10^{-6} mol)
mg	Milligram (10^{-3} g)
min	minutes
MeJA	Methyl jasmonate
Met	Methionine
mM	Milimole (10^{-3} mol)
N	Number of observations
nahG	Gene encoding salicylate hydroxylase
NahG	Plant line expressing *nahG*
NaOH	Sodium hydoxide
nm	nanometre
ns	Not significant
npr1	PR-1 non-expressor
NUV	Near ultra violet
O_2	Oxygen
P	Probability
PAL	Phenylalanine ammonia lyase
PCAF	Picro-cupric-ammonium formate
PDA	Potato dextose agar
kPa	Kilopascal
PDF1.2	Plant defensine 1.2
Pers. comm.	Personal communication
PG	Polygalacturonase
6-PGD	6-phosphogluconate-dehydrogenase
PL	Pectin lyase
PME	Pectin methyl esterase
PR	Pathogenesis related protein

LIST OF APPENDICES

®	Registered trade name
R	Resistant
RH	Relative humidity
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
sec	Second
SEM	Standard error of mean
STS	Silver thiosulphate complex
TMV	Tobacco mosaic virus
UV	Ultra violet
viz.	That is
VPD	Vapour pressure deficit
WCI	Wheat chemically induced
WR3	Wound responsive
≥	Greater than or equal to
≤	Less than or equal to
%	Percent
±	Plus or minus

CHAPTER 1

GENERAL INTRODUCTION

1.1 CUT FLOWER TRADE IN THE EUROPEAN UNION (EU)

The cut flowers industry is competitive with EU sales growing continuously in recent years (Kambil and van Heck, 1996; Anonymous, 2001). In 1999, total EU sales of flowers was estimated at about US$ 13 million (Table 1.1).

Table 1.1: EU sales of cut flowers and foliage between 1996 and 2003 in US$ million. Source: Flower Council of Holland (1999, 2000).

Country	**Year**				
	1996	**1997**	**1998**	**1999**	**2000**
Germany	3,983	3,478	3,494	3,343	3,492
Italy	2,025	2,001	2,101	2,152	2,557
France	2,127	1,930	2,027	1,939	2,350
UK	1,439	1,628	1,803	1,908	2,197
Spain	746	667	732	1,187	936
The Netherlands	670	551	564	543	561
Belgium	483	443	561	435	504
Austria	457	417	417	382	460
Sweden	391	338	348	172	404
Denmark	241	218	223	207	287
Finland	277	223	221	201	232
Greece	178	163	157	163	200
Portugal	135	137	147	148	207
Ireland	73	68	82	98	105
EU (Total)	**13,224**	**12,262**	**12,771**	**12,637**	**14,492**

Germany dominates flower sales, followed by Italy, France and the UK. The Netherlands is the leading flower marketer in EU with output valued at about US$ 3.5 billion in 1999. Although the number of growers in The Netherlands is decreasing, companies are increasing in size (Kambil and van Heck, 1996). The Netherlands is also the main flower importer in the EU accounting for 58% of imports in 1999 (Anonymous, 2001).

The Netherlands is one of the major markets for developing countries due to its massive trading role in distributing imported flowers throughout Europe. The importance of developing countries as suppliers to the EU is demonstrated by the presence of Kenya, Colombia, Ecuador, Zimbabwe, Thailand, Zambia, India, South Africa, Turkey, Tanzania and Uganda among the top 15 supplying countries (Anonymous, 2001).

The Netherlands trade in floricultural products is based on the dominant marketing channel of a system of auctions (Kambil and van Heck, 1996; Elshof, 2000; Anonymous, 2001) (Plate 1.1). The largest share of flowers goes through these auctions. They were initially established by Dutch flower growers in order to sell their products. The auctions in The Netherlands function as a price-setting mechanism for flower trade. They developed into major distribution centres for both domestic and foreign grown products to the European markets (Elshof, 2000; Anonymous, 2001).

Plate 1.1: Photograph of flower trolleys in a refrigerated area within the Aalsmeer flower auction. (Source: Flower Council of Holland, 2001).

Local and export wholesalers are vital links in the distribution chain from growers to consumers (Anonymous, 2001). Wholesalers arrange for the products to be carried to their foreign-based clients. Flowers after they have been delivered to a wholesaler, are prepared for further shipping in the right form (e.g. in buckets with holding solutions), quantity and packaging. Traditional florists still dominate the distribution at the retail level of flowers in most EU countries. The importance of retail supermarkets has been growing in recent years (Table 1.1). Growers and traders exporting cut flowers to the EU send their merchandise either to a wholesaler or to an auction in The Netherlands (Elshof, 2000; Anonymous, 2001) (Figure 1.1).

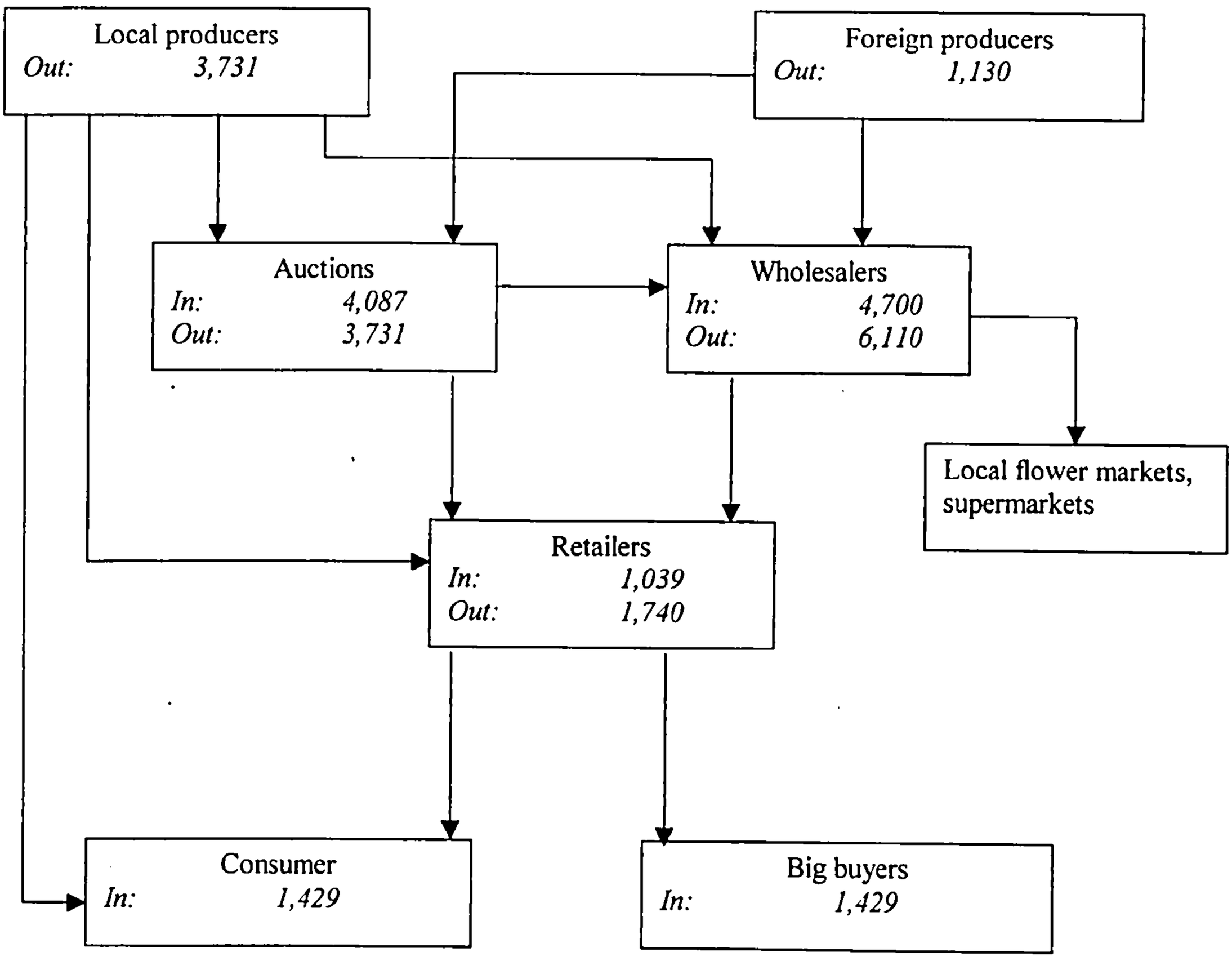

Figure 1.1: Distribution of cut flowers in The Netherlands in 1999. Numbers represent million NLG. (Source: Horticulture Commodity Board, 2001, The Netherlands).

In this respect, Netherlands auctions play a crucial role in the trade of flowers destined for both the domestic and other European markets. Products handled by import wholesalers are either sold on directly to a wholesale buyer or are submitted for auction. Export wholesalers re-export flowers to other EU member countries where they find their way to wholesalers and then retailers in other EU countries. Products sold at the auctions are bought by both local wholesalers and export wholesalers (Anonymous, 2001).

1.2 BACKGROUND

The freesia (*Freesia hybrida*) flower is a South African native species (Salinger, 1985). Freesias are commonly grown in The Netherlands and sold as cut flowers through the distribution chain outlined earlier. The high commercial importance of freesia cultivation is demonstrated by the large numbers of stems sold in Dutch auctions (Appendix 1.1, Table A1.2).

Botrytis cinerea Pers. is a common fungal pathogen that infects glasshouse grown ornamental crops under cool and humid conditions (Elad, 1988). In contrast to rose (Elad, 1988; Elad *et al.*, 1993a) and gerbera (Salinas and Verhoeff, 1995), there has been no published research on *B. cinerea* infecting freesia flowers. In recent years growers and sellers have been very concerned over problems related to infection of freesia by *B. cinerea* (D. Zwetsloot, pers. comm., 2000). Large quantities of *B. cinerea* infected cut freesia flowers grown in The Netherlands are rejected by UK wholesalers and retailers. Rejections result in economic losses that affect both growers and importers and strain cooperation. This project was conducted in collaboration between Cranfield University and Zwetsloots & Sons Ltd. Zwetsloots & Sons Ltd are located at Sandy, Bedfordshire, UK and were established in 1932. Quickly they became the UK's market leader in the supply of cut flowers with turnovers of £55 million. Zwetsloots & Sons Ltd are major wholesalers in the UK importing flowers from mainly The Netherlands and Spain and other countries outside the EU such as Kenya and Israel.

1.3 AIM AND OBJECTIVES

Infection of freesia flowers occurs inside the glasshouses where freesia crops are grown. However, visible symptoms of the disease appear during postharvest handling chain under favourable conditions for the pathogen (Salinas and Verhoeff, 1995). The general aim of this study was to control postharvest infection of cut freesia flowers by *B. cinerea.* The objectives were firstly to evaluate the effects of pre- and postharvest environmental conditions on *B. cinerea* disease incidence. Factors potentially affecting freesia infection were simulated and further evaluated in the laboratory. This work was supplemented with histological studies on the infection process.

The second objective was to control postharvest *B. cinerea* infection by inducing freesia defence responses. For this reason, novel plant defence activators such as the chemicals acibenzolar-S-methyl and methyl jasmonate and abiotic (i.e. UV-C irradiation) and biotic (i.e. *Aureobasidium pullulans*) agents were tested. Pre- and/or postharvest applications of acibenzolar-S-methyl and methyl jasmonate were tested. Novel chemical or biological treatments will increase flower resistance to postharvest infection by *B. cinerea* Infection may be suppressed if a stronger host hypersensitive reaction was induced. This may restrict pathogen development in smaller areas inside freesia tissue. It follows that, visible lesions would be reduced and therefore rejections avoided.

1.4 THESIS STRUCTURE

The literature on freesia flower characteristics, flower post-harvest handling, *B. cinerea* biology and plant defence physiology is reviewed in Chapter 2. The effects of pre- and post-harvest environmental conditions on post-harvest *B. cinerea* disease incidence is described in Chapter 3. The importance of environmental conditions was examined during real post-harvest handling and during simulation in the laboratory. In Chapter 3, histology studies on the freesia infection process by *B. cinerea* are presented. The efficacy of acibenzolar-S-methyl, methyl jasmonate and UV-C irradiation applied postharvest to suppress *B. cinerea* was tested and reported in Chapter 4. Chemical

concentration, application method, *B. cinerea* inoculum level and post-inoculation temperatures were the main factors examined. In Chapter 5, the effects of acibenzolar-S-methyl, methyl jasmonate and *Aureobasidium pullulans* on infection of freesia flowers by *B. cinerea* were tested preharvest in glasshouse flower-bed and pot trials. In the same Chapter, the effects of chemical concentration, fressia variety, and incubation temperatures on *B. cinerea* infection of cut freesia flowers were also examined. Finally, in Chapter 6, overall conclusions based on the results of this study and opportunities for future research are discussed.

CHAPTER 2

LITERATURE REVIEW

2.1 FREESIAS, CUT FLOWER TREATMENTS AND HANDLING

2.1.1 Freesia characteristics and cultivation

The freesia (*Freesia hybrida*) is a cut flower belonging to the family Iridaceae. It originated from several South African species. Freesias have attractive colour and shape and generally are sweetly scented (Plate 2.1) (Salinger, 1985).

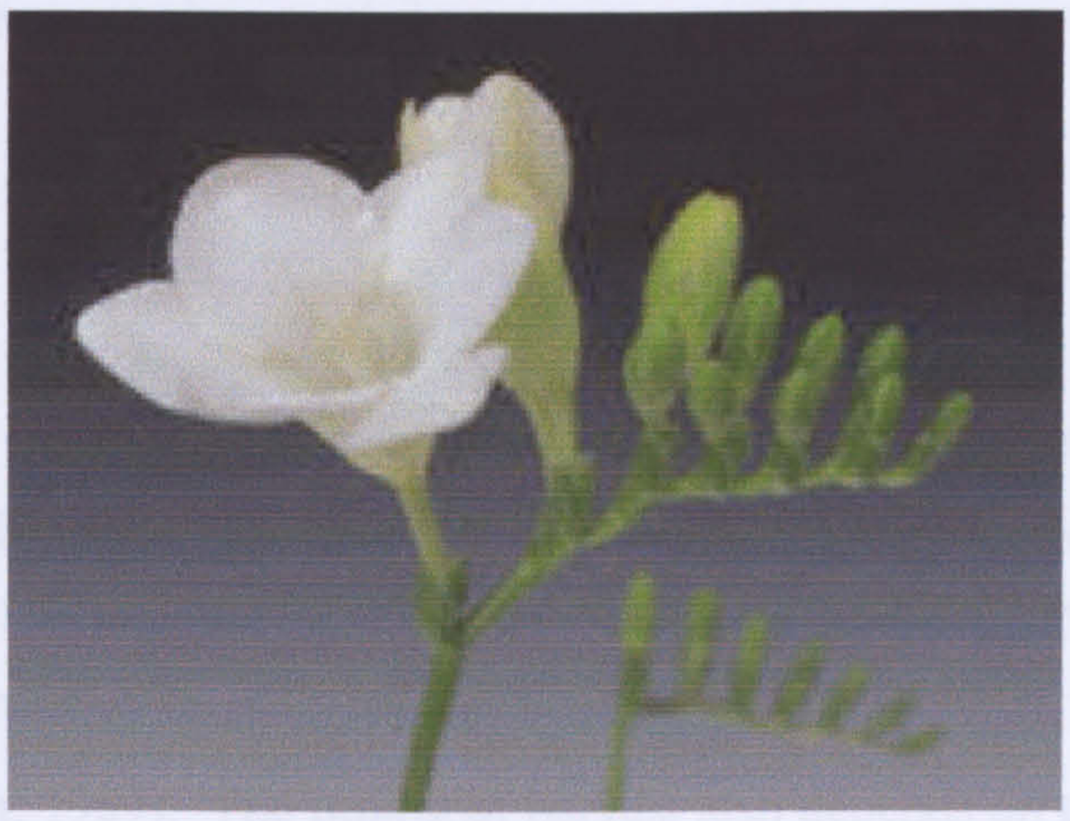

Plate 2.1: Freesia var. 'Elegance' inflorescence.

Cut freesia flower production is usually from corms, although it is also possible to grow flowering plants from seed. When the corm grows, a single shoot develops from its apex. The shoot has sheath leaves at its base. The flower stem (rachis) emerges from between these leaves. The rachis usually has a main terminal flower cyme, with two or more lateral cymes lower down the stem. The main flower stem fetches the highest retail

price, with laterals having less value. Under natural habitat conditions, plants flower in cool weather in late winter or by the beginning of spring (Salinger, 1985).

Temperature is the main factor affecting freesia growth and flowering. Corms need at least 16°C to grow, while seeds need temperatures over 20°C (Salinger, 1985). Inflorescence development takes place after the formation of about six leaves at an optimum temperature of 13°C. Above 18°C, inflorescence emergence can be inhibited. Inflorescence growth is slow below 9°C. Once an inflorescence is formed, optimal growth occurs at temperatures of 12-20°C (Salinger, 1985).

Light is the second most important factor for the growth of freesia crops. Freesias do not have a high light requirement, and even mid-winter day length, is enough to provide sufficient irradiation. Shading can be provided in conjunction with ventilation if conditions in late autumn or early winter are too warm and sunny.

2.1.2 Harvest and postharvest characteristics

2.1.2.1 Harvest

Longevity of some cut flowers (e.g. vase life) is associated with reserve carbohydrate levels (Rogers, 1962). In this context, flowers harvested late in the afternoon are likely to last longer than those cut early in the morning. This proposition is confirmed for some flowers such as roses, but not for others such as gerberas (El-Gamassy and El-Fattah, 1969). The timing of harvest also depends on the desired flower development stage, and is specific for individual species. During freesia flower development, glucose, fructose and sucrose content increases 15–20 fold with maximum concentrations being reached during anthesis (van Meeteren *et al.*, 1995). In harvested freesia flowers, the concentration of sugars in the 5^{th} floret from the base of inflorescence were only 20% compared to 5^{th} floret of inflorescence attached on the plant (van Meeteren *et al.*, 1995).

Harvest is influenced by season, prevailing environmental conditions, distance to market and consumer preferences. Flowers are generally cut at the earliest stage of

development where subsequent bud opening can be assured (Halevy and Mayak, 1981a; Goszczynska and Rudnicki, 1988). Harvest at an early stage has the advantages of greater resistance to detrimental handling conditions, ethylene, mechanical injury and pathogen infection (Maxie *et al.,* 1973). Flowers like freesia, rose, gladiolus and iris are usually harvested at the bud stage and continue to develop when stood in water solutions after harvest. However, if flowers are harvested premature, flower development may fail to occur (Halevy and Mayak, 1981a). The ideal freesia maturity stage at harvest is a factor that determines bud opening with bud carbohydrate status influencing bud opening after harvest (Sytsema-Kalkman *et al.,* 1995). Other flowers such as carnations, gerberas, orchids, anthuriums, dahlias and chrysanthemums do not develop to a commercial stage in water after harvest (Goszczynska and Rudnicki, 1988). For local sales, freesias are harvested when the first flower of the spike is at bud stage. For distant sales, harvest of freesias takes place when the first bud of the spike has just become coloured. The primary freesia flower stem is harvested first when it reaches a length of approximately 70 cm (Salinger, 1985).

2.1.2.3 Storage

Storage is important for maintaining the quality and longevity of cut flowers after harvest (Halevy and Mayak, 1981a). Wet and dry storage are both commonly used methods for flower storage (Nichols, 1967; Lutz and Hardenburg, 1968; Nichols, 1971; Nowak and Rudnicki, 1979). Wet stored flowers are kept for a relatively short period of time with their stems standing in water or vase solution. Dry storage is used for longer storage periods. Certain flowers like freesia and dahlia maintain their quality for longer periods when stored wet. Freesia var. 'Ballerina' flowers had a vase life of 7 days when previously stored wet and only 2 days when stored dry at 0-1°C (Goszczynska and Rudnicki, 1988).

Low temperature is the main factor for maintaining flower quality after harvest and during storage (Goszczynska and Rudnicki, 1988). Storing cut flowers at low temperatures reduces respiration rate, metabolic activity (Reid and Kofranek, 1980), ethylene production (Nichols, 1966) and fungal growth (Hammer and Marois, 1989;

Joyce, 1993; Taylor *et al.,* 1997). Storage temperatures for temperate flowers like freesias, roses, chrysanthemums and carnations can be close to the freezing point of the tissue (Halevy and Mayak, 1981a). However, tropical flowers like tropical orchids and anthuriums can be chilling sensitive. Therefore, they need higher temperatures between 10 and 15°C during storage. Temperature during storage can be crucial for kangaroo paw cut flowers (Joyce and Shorter, 2000). At 0°C kangaroo paw cut flowers suffered chilling injury and damage in the soft stem region below the flower. Deterioration was also evident at 10°C. Storage of kangaroo paw cut flowers at 0°C significantly reduced vase life compared to unstored flowers (Joyce and Shorter, 2000). However, freesia flowers can be stored at 0-0.5°C for up to 14 days (Gast, 1997).

Humidity control is also important during storage (Halevy and Mayak, 1981a). Moisture loss in stored flowers is directly related to the vapour pressure deficit. Humidity should be maintained at high levels, but without causing water condensation on the flower surface. Thus, relative humidity should be kept at 90-95% (Lutz and Hardenburg, 1968). At constant high relative humidity levels (i.e. 95-100%), free water may form on the surface of flowers. This condensation can result in infection by fungal pathogens such as *B. cinerea.* Infection symptoms caused by *B. cinerea* are therefore observed after storage or transport at low temperatures, under high humidity regimes or temperature fluctuations (Elad, 1988). Cold storage of three rose cultivars at reduced humidity levels between 50 and 80% markedly reduced *B. cinerea* disease severity (Hammer and Marois, 1989).

Controlled atmosphere (CA) storage has been proposed for long-term storage of cut flowers (Rogers, 1973). CA is applied to inhibit ethylene production and reduce respiration rates of ornamentals. Carbon dioxide also acts as a competitive inhibitor to ethylene for ethylene binding sites when it is present at high concentrations inside the storage room (Halevy and Mayak, 1981a). Recommended temperatures and O_2 and CO_2 concentrations for CA storage vary between flower species (Goszczynska and Rudnicki, 1988). CA storage for cut freesia flowers is optimum at 10% CO_2 and 21% O_2 at 1-2°C (Goszczynska and Rudnicki, 1988). These conditions allow a maximum storage period for freesia of 3 weeks.

Cut flowers can also be stored in chambers under decreased atmospheric pressure (hypobaric storage). Hypobaric storage was found to be effective at decreasing ethylene

and CO_2 accumulation in apples (Burg and Burg, 1965; Burg, 1966). Gas exchange between plant tissue and atmosphere occurs predominately through stomata and/or lenticels. This exchange is enhanced when the commodity is below atmospheric pressure (Burg and Burg, 1965). Gas diffusion outwards is facilitated because the density of the external gas environment is lowered. If atmospheric pressure drops by 0.1 atm volatiles escape the tissue 10 times faster. Dilley (1977) showed that carnations kept for 5 weeks under hypobaric storage produced less ethylene than fresh flowers. Other factors like temperature, humidity and CO_2 concentration can also play a substantial role in modulating the effects of hypobaric storage.

2.1.2.4 Packing

Packaging provides a protective barrier against mechanical damage and unfavourable environmental conditions during storage and distribution. The effects of temperature fluctuations within different package designs for cut roses infected by *B. cinerea* have been reported (van der Sman *et al.*, 1996). An increase in *B. cinerea* disease incidence on cut roses was observed after re-warming of flowers inside the packing box. Size and location of ventilation holes of the box had an effect on infection levels. With increasing relative humidity inside model packages postharvest infection of Geraldton waxflowers by *B. cinerea* increased linearly at 20°C (Taylor *et al.*, 1997). Wrapping rose flowers in cellophane sleeves before reducing humidity in a storage room decreased water loss but impaired *B. cinerea* control (van der Sman *et al.*, 1996). Problems with fungal disease may occur in the distribution chain when packed produce experiences varying temperature regimes. Temperature fluctuations that result in condensation of water vapour onto flowers can stimulate spore germination.

2.1.2.5 Transport

Flowers have traditionally been grown close to retail centres where they are delivered within few hours after harvest (Halevy and Mayak, 1981a). However, long

distance transport of cut flowers has become prevalent with the evolution of improved handling, transport and cooling methods. Transportation of cut flowers over long distances has been achieved by air (Farnham *et al.*, 1979). However, increases in the costs of airfreight encourage alternative transportation such as by land or sea (Farnham *et al.*, 1979). Problems with long-term transport of cut flowers are similar to those occurring during storage. These involve bud opening during transit, petal and foliage drying, disease spread, failure of buds to open after transport and reduction in vase life (Halevy and Mayak, 1981a). Pre-cooling takes place immediately after harvest in order to remove field heat and maintain low respiration rates (Halevy and Mayak, 1974). Special treatments (i.e. pulsing) after harvest are also applied to flowers preceding long-term transport.

2.1.2.6 Involvement of plant hormones in cut flowers postharvest life

Ethylene

Ethylene (C_2H_4) is the plant hormone principally responsible for plant tissue senescence. Ethylene synthesis in plant tissue depends on methionine and is accomplished via the biosynthetic pathway shown in Figure 2.1. The rate-limiting step in this pathway is the conversion of AdoMet to ACC. This step catalysed by the enzyme ACC synthase (Fluhr and Matto, 1996) (Figure 2.1). The final step, the conversion of ACC to ethylene, requires oxygen and is catalysed by the enzyme ACC oxidase (Fluhr and Matto, 1996). Flowers produce ethylene after harvest. Ethylene production by some cut flowers species follows a typical climacteric pattern consisting of three phases (Yang, 1980). The first stage is ethylene production at low steady levels; the second stage is an accelerated rise in ethylene production and the third stage is a decline. In climacteric flowers such as 'White Sim' carnation, the senescence co-ordinating effects of ethylene are visible when flowers pass through the second stage. Ethylene is produced in all parts of the bloom, although its production rate varies among organs (Yang, 1980). For example, the highest ethylene emanation was detected in carnation style and petals (Nichols, 1977).

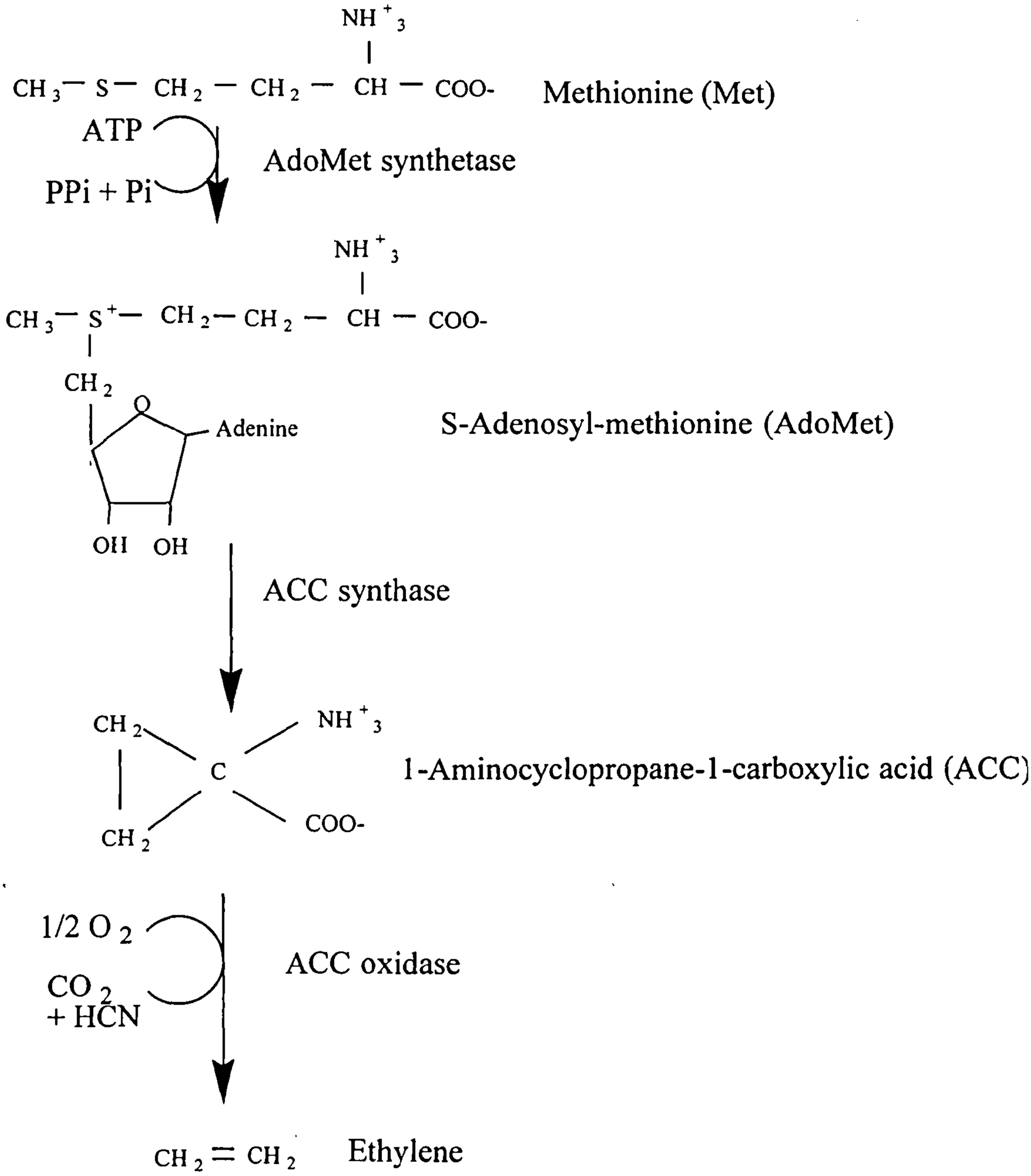

Figure 2.1: Ethylene biosynthesis from methionine (redrawn from Taiz and Zeiger, 1998).

It has also been suggested that flower tissues sensitivity to ethylene depends on tissue maturity (Mayak and Kofranek, 1976). Exposure of mature carnation flowers to ethylene resulted in visible senescence symptoms. In contrast, exposure of young carnation

flowers to ethylene resulted in temporary aging symptoms, which disappeared after ethylene removal (Mayak and Kofranek, 1976). Ethylene is present in small quantities inside immature flower tissue. Levels increase after auto-stimulation during the rapid senescence phase. The sensitivity of flower tissue to ethylene can change as a result of complex interactions among internal factors such as other plant hormones, like abscisic acid (Halevy and Mayak, 1981b). Experiments have demonstrated the tight interaction between senescence of plant tissue and ethylene production (MacRobbie, 1971; Kende and Baumgartner, 1974; Hanson and Kende, 1975). Senescence includes processes leading to cell disorganization. These involve disintegration of cell membranes and uncontrolled mixing of vacuolar contents and cytoplasm (Halevy and Mayak, 1981b). Vacuolar substrate leakage as a result of increased membrane permeability could be a precondition for the rise in ethylene production (Halevy and Mayak, 1981b). However, some experimental evidence contradicts this proposal. Suttle and Kende (1978) did not observe any pigment leakage in *Tradescantia* petals during the ethylene rise. Moreover, changes in membrane permeability following ethylene production in *Tradescantia* petals had a lag of 2h (Suttle and Kende, 1980). Furthermore, increase in membrane permeability of senescencing rose petals is accompanied by a massive loss in phospholipid. Accordingly, ethylene may exert its effects through enhanced phospholipid loss (Borochov *et al.*, 1978).

Controlling ethylene inside storage rooms can prolong cut flower longevity (Halevy and Mayak, 1981b). Protection of stored flowers against the detrimental effects of ethylene can be achieved in different ways. Ventilation of the storage room with fresh air can prevent ethylene accumulation (Rogers, 1973; Halevy and Mayak, 1981b; Goszczynska and Rudnicki, 1988). Absorption of ethylene by filtration devices filled with strong absorbants such as brominated activated charcoal (Smock, 1944) or addition of small packets containing potassium permanganate absorbed on a silicate carrier (Liu, 1970) can also be effective in removing ethylene. Ethylene production by plant tissue can be prevented if oxygen (O_2) is held at very low levels, since oxygen is an essential element for ethylene production (Burg and Thimann, 1959). The concentration of carbon dioxide (CO_2) in the storage environment can also affect ethylene accumulation (Halevy and Mayak, 1981b). Carbon dioxide can act as a competitive inhibitor of ethylene binding due to its molecular similarity in size and structure (Καρατάγλης, 1999).

Temperature has also been reported to play an important role in ethylene production by carnation flowers (Barden and Hanan, 1972). Fischer (1950) noted that ethylene evolution was greatly reduced when temperature was dropped below 4.4°C.

2.1.2.8 Chemical treatments for prolonging vase life

Enhancement of cut flower senescence is often related to a decrease in water content of the flower tissue, an increase in ethylene production and action, a decrease in carbohydrate levels and a reduction of water uptake (Rogers, 1973; Goszczynska and Rudnicki, 1988). Various techniques, many of them involving chemical treatments are used for preservation and promotion of cut flower quality.

Conditioning or hardening restores the turgidity of flowers suffering water deficit from handling in the field or in the glasshouse or during storage or transportation (Halevy and Mayak, 1981a). For conditioning, stems can be stood initially in warm water at room temperature and then overnight in cooler water (Rogers, 1963). When flowers are wilted, immersing the entire flower in water for 1h prior to the normal conditioning procedure can rapidly restore lost turgidity. Conditioning is preferably done with deionized water containing a germicide, but without any sugar addition (Lancaster, 1975).

Pulsing is the process used for flowers that are to be either stored or exported (Kofranek, 1976; Halevy and Mayak, 1981a; Goszczynska and Rudnicki, 1988). Pulsing is commonly practiced on immature harvested flowers. It involves standing stems in aqueous solutions that usually contain sucrose together with other compounds such as biocides, anti-ethylene agents, growth regulators and weak acids (Kofranek, 1976; Halevy and Mayak, 1981b; Goszczynska and Rudnicki, 1988). Ingredients in pulsing solutions usually differ among species and even varieties (Halevy *et al.*, 1978). For example, gladiolus is pulsed with a solution containing 20% sucrose (Mayak *et al.*, 1973), whereas carnations (Halevy and Mayak, 1974) and *Gypsophila* (Farnham *et al.*, 1979) require 10% sucrose. Roses require 2 to 5% sucrose in the pulsing solution (Halevy *et al.*, 1978). Addition of sucrose to the pulsing solution can result in retardation of senescence as simple carbohydrates like sucrose, can delay protein breakdown and maintain membrane integrity including the structure and function of mitochondria

(Halevy and Mayak, 1981a). Moreover, exogenous sugar can inhibit ethylene production (Dilley and Carpenter, 1975) and decrease susceptibility of carnations to exogenous ethylene (Mayak and Kofranek, 1976). Sugar also improves the water balance of cut flowers, sometimes by closing stomata and thereby reducing transpirational water loss. Accumulation of sugar inside the cells can increase the osmotic pressure and lower the osmotic potential. The later improves the ability of tissues to absorb more water and thus better maintain turgidity (Acock and Nichols, 1979).

Vase life and storage longevity can be affected by the growth of microorganisms in holding solutions (van Doorn and Peric, 1990). Microorganisms reduce the longevity of cut flowers by plugging stems (organic occlusion). Organic occlusion is usually caused by accumulation of living and/or dead bacteria that obstruct water flow to the upper parts of the flower. Moreover, toxic metabolites produced by bacteria may induce ethylene production (Van der Molen *et al.*, 1983). Bacterial growth in vase solutions is prevented by bactericides such as silver nitrate ($AgNO_3$), 8-hydroxyquinoline citrate or sulfate (8-HQC or 8-HQS), aluminum sulfate [$Al_2(SO_4)$], sodium hypochlorite, copper sulfate, aluminum nitrate, silver nitrate, and zinc acetate (Rogers, 1973). Silver nitrate greatly extends the longevity of chrysanthemum, gerbera, gladiolus, carnation, statice, china ester, Phalaenopsis, cattleya orchids and bougainvillea (Halevy and Mayak, 1981b). Beyer (1976) found that silver nitrate also had anti-ethylene activity. 8-hydroxyquinoline citrate and sulfate have extensively been used as broad-spectrum bactericides (Halevy and Mayak, 1981b). 8-HQC and 8-HQS produce an unfavourable environment for microbial growth by reducing the acidity of the holding solutions (Rogers, 1973; Halevy and Mayak, 1981b; Goszczynska and Rudnicki, 1988; van Doorn and Perik, 1990). Vascular blockage in cut rose stems was effectively reduced by HQC treatment in low pH solutions (van Doorn and Perik, 1990). Joyce (1988) found that 8-HQS at 200 mg L^{-1} plus 1-3% w/v sucrose in vase solutions increased the vase life of Geraldton waxflower.

The detrimental effects of ethylene can be prevented with anti-ethylene agents in the vase solutions. The silver thiosulphate complex (STS) has shown effective in inhibiting both the action and autocatalytic production of ethylene (Veen, 1979). STS acts by blocking ethylene-binding sites reducing temporarilly ethylene effects (Serek and Sisler, 2001). In addition, STS treatment protects ethylene-sensitive flowers from abscission. For example, STS treatments of Geraldton waxflower reduced ethylene-

induced flower abscission (Joyce, 1993). Silver thiosulphate pulsing for 15 min with 4 mM Ag^+ or overnight with 0.5 mM Ag^+ were both effective treatments (Joyce, 1988). Inflorescences of freesia vars. 'Oberon' and 'Ballerina' treated with STS had greater fresh weight, water uptake, water holding capacity, floret opening and vase life compared to water treated inflorescences (Amariutei *et al.*, 1995). Solutions containing only STS with no added sucrose or bactericide doubled the vase life of carnations (Reid *et al.*, 1980). STS treatments also prevent leaf and petal abscission in various other cut flowers and pot plants (Veen, 1983). Small beneficial effects from STS treatments have been shown for anthurium (Paull and Goo, 1982), gladiolus (Farhoomand *et al.*, 1980) and tulip (Nichols and Kofranek, 1982). Increased concentrations above a threshold level of Ag^+ applied as STS on harvested Geraldton waxflowers gave no improvement in vase life (Joyce, 1988).

As STS contains the heavy metal silver, it is a potential environmental pollutant, and its commercial use is under scrutiny in some countries. The novel gaseous ethylene inhibitor 1-methylcyclopropene (1-MCP) has been tested as an alternative to STS for potted flowering plant and cut flower storage life extension (Serek *et al.*, 1994; Macnish *et al.*, 1999; Macnish *et al.*, 2000). The ethylene inhibitory effects of 1-MCP treatment increased by increasing 1-MCP concentration by up to 20 nL L^{-1} whereas flower protection was equal to that achieved by spraying with STS at 0.5 mM (Serek *et al.*, 1994). In grevillea and waxflower cut flowers, 1-MCP prevented flower abscission when flowers were exposed for 1 day to ethylene and stored at 2 or 20°C (Macnish *et al.*, 2000). Reduction of the ethylene effects on *Boronia heterophylla* flowers treated with 1-MCP were reported by Machish *et al.* (1999). However, there is concern on flowers quickly regaining sensitivity to ethylene after 1-MCP treatment (Blankenship and Dole, 2003). According to Muller *et al.* (2000), this happens because more ethylene sites are produced during further plant development. Only repetitive 1-MCP treatment can confer protection from ethylene effects (Blankenship and Dole, 2003). In contrast, STS (silver ion) can remain in the plant tissue for longer time after synthesis of new ethylene binding sites continuously inactivating ethylene response (Serek and Sisler, 2001)

2.2 INTRODUCTION TO ORNAMENTAL INFECTION

2.2.1 Common freesia diseases

Freesia plants can be infected by a limited number of pathogens. Fungal pathogens found in California include the *Fusarium* spp. which cause corm and leaf wilt (Anonymous, 1960). *Stromatinia gladioli* is sometimes corm-borne, but infection often results from growing freesias on land already contaminated with the pathogen from previous freesia or gladiolus crops. The imperfect stage of this fungus is known as *Sclerotinia gladioli. Didymella macrospora* is a fungus that causes spots on freesia leaves. The imperfect stage of this fungus is *Heterosporium iridis. Penicillium gladioli* causes postharvest corm-rots. The above-mentioned fungi usually infect freesia crops in the field. The most important disease affecting postharvest freesia quality is *Botrytis cinerea,* causes flower specking. This pathogen also attacks the foliage and dead leaves under humid, cool conditions (Anonymous, 1960). *B. cinerea* can also damage harvested freesia flowers during storage and transportation and thereby reduce their quality.

2.2.2 *Botrytis cinerea* infecting ornamentals

Botrytis cinerea Pers. is the conidiaphore anamorph of *Botryotinia Fuuckeliana* (de Bary) Whetzel (Jarvis, 1980a) belongs to the Class Deuteromycetes and the Phylum Ascomycota. In The Netherlands, gerbera production has suffered in recent years due to *Botrytis* infection problems that often lead to serious economic losses (Salinas, 1992; Salinas and Verhoeff, 1995). *B. cinerea* is also pathogenic to Geraldton waxflower (Joyce, 1993; Tomas *et al.*, 1995). Flowers artificially inoculated with *B. cinerea* suffered increased abscission from their pedicels. *B. cinerea* attacks rose flowers producing necrotic spots or blister-like patches on petal surfaces (Pie and De Leeuw, 1991; Williamson *et al.,* 1995). Infection of rose flowers by *B. cinerea* was described by Elad (1988) as restricted brown lesions. *B. cinerea* also damages phylloclades of ruscus by causing small, dark water soaked necrotic lesions encircled by a faint halo. These lesions later become brown without growing in size (Elad *et al.*, 1993b). *B. cinerea*

disease symptoms on geranium flowers have been described by Strider (1985) as flower blight, leaf blight and stem rot. There is a lack of published studies on freesia flowers infected by *B. cinerea.*

Infection of gerbera flowers by *B. cinerea* occurs inside the glasshouse during crop cultivation, but symptoms may develop during storage or transport following fluctuations in temperature (Salinas and Verhoeff, 1995). Conditions of temperature and RH favourable for the pathogen results in rapid disease development after harvest (Salinas *et al.*, 1989). According to Salinas *et al.* (1989), high RH regimes enhance its aggressiveness. Disease caused by *B. cinerea* on gerbera ray florets is often observed as small necrotic fleck lesions 'spots'. Similar symptoms developed in the laboratory under controlled conditions after artificial inoculation of gerberas with the fungus at temperatures ranging from 4 to 25°C (Salinas and Verhoeff, 1995).

2.3 THE BIOLOGY OF *BOTRYTIS* DISEASES

2.3.1 Morphology

B. cinerea mycelium has typical characteristics of the Phylum Ascomycota (Jarvis, 1977). Extension from the germinated spore occurs at the hyphal apex and growth of the hyphae increases over time. Septa are frequently observed and are perforated by a simple pore. The conidiophores are tall, dark-coloured and irregularly or dichotomously branched (Plate 2.2). Near the apex of each conidiophore, a number of short, dark septated branches develop. Each of these branches has a terminal ampulla on which conidia are formed. The conidia are hyaline or pigmented, ellipsoid to obvoid - globoid, usually continuous, with 1-3 septa (Ellis and Walter, 1974). Conidia germinate in nutrient solutions and less readily in water to usually form 1 to 5 germ tubes (Jarvis, 1977).

Plate 2.2: *Botrytis cinerea* conidiophores and typical terminal conidial bunches with characteristic morphology of the pathogen cultured on PDA (x 20).

2.3.2 Life cycle

2.3.2.1 Sporulation and dispersal

The importance of light quality in promoting *B. cinerea* sporulation has been studied. Green, yellow and red light significantly promoted *B. cinerea* germ tube elongation compared to near ultra violet (UV-A), white, blue and far-red light (Islam *et al.*, 1998). *B. cinerea* sporulation was facilitated under continuous UV-A in comparison to continuous darkness (Leach 1962). Tan and Epton (1973) examined the effects of light on *B. cinerea* sporulation on culture media, considering also other factors such as the age of spore inoculum, temperature, spore density and mycelial growth. They found that exposure of *B. cinerea* cultures to black light (UV-A), promoted conidial formation.

Moreover, culture age, duration of exposure and intensity of black light had significant effects on sporulation. *B. cinerea* conidia formation was suppressed when cultures were exposed for 12h to black light following exposure to blue light (Tan and Epton, 1974).

Dispersal of *Botrytis* conidia is mainly through airflows and water splashes (Jarvis, 1977). Pady and Kelly (1954) demonstrated the presence of *Botrytis* conidia in the air over the Arctic and Atlantic Oceans. This was done by exposing Petri dishes containing nutrient agar from an aircraft. At lower altitudes (i.e. ground level) a large number of *Botrytis* conidia has been found in urban areas especially during summer and autumn (Pady and Kapica, 1956). Sreeramula (1959) found a diurnal periodicity of *Botrytis* spores trapped from the air. *Botrytis* spore numbers peaked at midday at a concentration of 400 spores m^{-3}.

Other factors can affect the liberation and dispersal of inoculum (Lucas, 1997). Insects such as bees, aphids, beetles and leafhoppers living in the crop can disperse pathogen populations from one plant to another. Human activities around crops may also influence pathogen dispersal.

B. cinerea spore dispersal mechanisms were first described by Bary (1884). The mature conidiophore was described as a flattened, twisted ribbon that responds to changes in RH. During the violent twisting of the mature conidiophore about its long axis, spores were dislodged by centrifugal force. Jarvis (1962b) suggested two individual processes. Firstly, spore release as a result of hygroscopic mechanisms, and secondly, spore dispersal by other agents of wind, water splashes and vectors. Two diurnal periods when prolific dispersal was correlated with the changes in RH levels were proposed. The first peak of conidial dispersal was observed from mid-morning until noon, when humidity levels dropped from 85 to 65%. The second peak was during evening hours, when RH rose from approximately 65 to 85%. These field observations were confirmed under laboratory conditions, when spore release and dispersal occurred after a 5% fluctuation in RH (Jarvis, 1960).

2.3.2.2 Conidial germination

Upon a compatible interaction between a host and a pathogen, disease develops following conidial germination, penetration and colonization of the host (Kamoen, 1992). Toxic substances released by the plant and/or competition/antagonism by phylloplane microflora can inhibit germination of *B. cinerea* conidia. Germination can be inhibited due to nutrient competition between conidia (Kamoen, 1992). Conidial germination is self-inhibited when spores are present in an inoculum droplet at high density (Last, 1960). On the other hand, conidial germination is stimulated by addition of nutrients and/or pollen to infection droplets (Mansfield, 1980).

Enzymes and toxins are secreted by the pathogen during germination and penetration (Kamoen *et al.,* 1980; Verhoeff, 1980; Elad, 1997). Glucans secreted from *B. cinerea* are not particularly toxic to plant cells and they do not act as elicitors for antifungal compound production by the host (Elad, 1997). On the other hand, cutinases secreted by *B. cinerea* have host specificity and play an essential role in the penetration process (Elad, 1997). Cutinases degrade cutin in the plant cuticle. Such cutinolitic activity has been detected in *B. cinerea* conidia growing on culture media (Salinas, 1992). Gerbera flowers were protected from infection by *B. cinerea* with monoclonal antibodies against purified cutinases (Salinas, 1990). Pectinases are cell wall degrading enzymes secreted by conidia and young germ tubes (Kamoen, 1992). Pectinases have been found in germination fluid and play a substantial role in the penetration process (Verhoeff, 1980; Kamoen, 1992). Pectin-degrading enzymes described for *B. cinerea* include polygalacturonases (PGs) (Leone, 1990), pectin lyases (PLs) (Heale, 1992) and pectin methyl esterases (PMEs) (Marcus and Schejter, 1983).

2.3.2.3 Host penetration

Penetration of host epidermal cells by *B. cinerea* is achieved with a thin infection peg. This may arise from an appressorium or by the tip of a germ tube (Clark and Lorbeer, 1976; Rijkenberg *et al.,* 1980; Pie and De Leeuw, 1991; Salinas and Verhoeff,

1995; Williamson *et al.*, 1995). Direct penetration of epidermal cells of rose petals occurred by the formation of a small peg on the tip of the germ tube (Pie and De Leeuw, 1991). During penetration, the cuticle was further degraded by cell wall degrading enzymes such as pectinase (Verhoeff, 1980).

Wounds on the surface of the host generally favour penetration by *B. cinerea*. Wounds provide easy access and lower host resistance via cell injury and death. Necrotic cells provide a base from where the fungus may diffuse degrading enzymes and toxins into adjacent host cells (Kamoen, 1992). As a result, *B. cinerea* may live saprophytically on necrotic tissue (Jarvis, 1977).

2.3.2.4 Infection

Quiescence

B. cinerea conidia germinate on flower petals in water droplets and can penetrate the epidermis with or without infection structures (Jarvis, 1977). Infection can be invisible/latent (e.g. strawberries, grapes) until harvest. Symptoms develop during storage and/or transportation at low temperatures and high relative humidity (Elad, 1988). Infection is observed as either grey mould or hypersensitive flecking on the host. On gerberas, infection is visible as hypersensitive flecking or specking on the ray florets making flowers unsaleable (Salinas *et al.*, 1989). *Botrytis* diseases are generally characterized by a period of indefinite duration where the fungus is apparently inactive in a symptomless host (i.e. latent) or lesions are visible but not extending (non-aggressive, as opposed to aggressive when lesions are expanding) (Beaumont *et al.*, 1936). Swinburne (1983) defined quiescent infection as the inhibition of development of the pathogen through physiological conditions imposed by the host until some stage of maturation has been accomplished. This definition implies that disease development inhibition is due to metabolic host reactions. Powelson (1960) confirmed the symptomless presence of *B. cinerea* in strawberry flowers and fruit as he isolated the fungus from surface-sterilized tissue pieces. Jarvis (1962a) confirmed quiescent infection

of strawberry fruit and showed the same infection characteristics on raspberries. *B. cinerea* occurrence was greater in raspberry flower parts (i.e. petals, calyx) previously damaged by frost. The latent period (time from infection to symptom development) lasted up to 6 weeks depending on weather conditions. McClellan and Hewitt (1973) found that latent infection of grapes by *B. cinerea* occurred after infection of the stylar end of the flower and that it became aggressive, even in the absence of wet weather, when the berry reached maturity.

Colonization of the host

Lesion expansion is achieved either by chemical secretions that mortify the cells of leaves, stems, fruits, etc., as the fungus grows within necrotic tissue or as a result of hyphal growth and its establishment inside host cells such as those of strawberry flower petals. In tomato leaves, the *B. cinerea* infection process consisted of three phases: 1) the formation of primary necrotic lesions, 2) a quiescent phase, and 3) primary lesion expansion 72h after inoculation (Benito *et al.*, 1998). According to Kamoen (1972), *B. cinerea* hyphae do not parasitize healthy tissue but rather formerly healthy cells following secretion of hydrolytic enzymes such as glucanases, cutinases and pectins which macerate cell walls. Extensive work has been done on the isolation of substances secreted by the fungus and which cause cell death. The movement of these substances through host tissue is also under investigation. According to this model, diffusion of pathogen-derived secretions may occur via intercellular spaces. The distance over which cells are killed by fungal secretions is dependent on the water content of cells. Expansion increases at low water vapour deficits that result in an increase in intercellular water content (Kamoen, 1972).

2.3.3 Epidemiology

2.3.3.1 Temperature

Temperature is one of the main environmental factors affecting initial infection by the pathogen and, possibly, the transition from latent and non-aggressive infection into the aggressive state of the disease (Jarvis, 1977). Hennebert and Gilles (1958) found that the optimum temperature for conidial germination on artificially inoculated ripe strawberry fruit surfaces was 20°C. Conidial germination began within 90 min of inoculation and all conidia germinated after 5h. Grape flowers and berries were infected by *B. cinerea* in the optimum temperature range of 20.8-23.7°C (Nair and Allen, 1993). The optimum temperature range for *B. cinerea* mycelial growth was found to be 20-22°C (Brown, 1922; Adair, 1971). Conidial germination on gerbera ray florets occurred over a wide range of temperatures from 4-20°C (Salinas *et al.*, 1989). Temperatures ranging from 18-20°C were optimum for *B. cinerea* lesion development on gerbera flowers grown under glass in The Netherlands (Kerssies, 1994). Temperatures of 20 and 25°C inside gerbera climate chambers produced more *B. cinerea* lesions on gerbera ray florets than temperatures of 10 or 15°C (Kerssies, 1994). The speed of tomato stem rotting caused by *B. cinerea* was positively enhanced by increased temperatures (Shtienberg *et al.*, 1998). However, temperatures ranging between 18 and 25°C stopped *B. cinerea* spread on gerbera flowers due to an effective hypersensitive reaction (Salinas and Verhoeff, 1995). However, the fungus could be isolated from the infection site suggesting that it remained alive inside the host tissue. At temperatures higher than 30°C, conidial germination and lesion formation was inhibited (Salinas *et al.*, 1989).

Temperature is a particularly important factor in the speed of *B. cinerea* growth when combined with high RH (Table 2.1). In gerberas, 7h incubation at 18-23°C and 100% RH was sufficient for visible lesion development (Salinas *et al.*, 1989). Maintaining Geraldton waxflower at temperatures below 10°C and RH below 90% reduced postharvest disease severity, flower abscission and desiccation (Taylor *et al.*, 1997).

Table 2.1: Effect of temperature (°C) and relative humidity (%) on *B. cinerea* infection of ornamentals.

Host	Temperature (°C)	Humudity (%)	Result	Reference
Geraldton waxflower	<10	<90	Postharvest disease severity reduction	Taylor *et al.*, 1997
Gerbera ray florets	4-20	nm	Conidial germination	Salinas *et al.*, 1989
Gerbera flowers	18-20	nm	Lesion development	Kerssies, 1994
Gerbera flowers	18-25	nm	*B. cinerea* spread was stopped	Salinas and Verhoeff, 1995
Gerbera flowers	18-23	100	Lesion development	Jarvis, 1980
Gerbera flowers	Room-temperature	100	Necrotic lesions	Salina *et al.*, 1989
Gerbera flowers	Room-temperature	50-70	Not visible lesions	Salina *et al.*, 1989
Gerbera flowers	nm	nm	Positive correlation between lesion numbers and RH	Kerssies, 1993
In-vitro	20-22	nm	*B. cinerea* mycelial growth	Adair, 1971
In-vitro	20-22	nm	*B. cinerea* mycelial growth	Brown, 1922
In-vitro	20, 15 and 5	100	*In-vitro* conidial germination	Rippel and Heilman, 1930
Rose flowers	nm	nm	Positive correlation between lesion numbers and RH	Kerssies *et al.*, 1995
Rose flowers	15	70, 80, 90, 94, 97 and 100	Infection occurred only when RH was > 94%	Williamson *et al.*, 1995

nm: not mentioned

2.3.3.2 Humidity

As mentioned above, humidity is an extremely important factor for *B. cinerea* disease establishment (Table 2.1). Rippel and Heilman (1930) reported that 100% of *Botrytis cinerea* conidia germinated *in-vitro* at temperatures of 5, 15 and 20°C when RH was 100%. At 95% RH, 85% of *B. cinerea* conidia germinated at 15 and 5°C, but all germinated at 20°C. At 90% RH, 85% of conidia germinated at 20°C, but none at 5 and 15°C. RH regimes had significant effects on *B. cinerea* spore germination and lesion development *in planta* (Salinas *et al.*, 1989).

Incubation of artificially inoculated gerbera flowers at 100% RH at room temperature produced typical necrotic lesions after 7h. However, at 50-70% RH at room temperature symptoms were not evident (Salinas *et al.*, 1989). Positive correlations between lesion numbers on gerbera (Kerssies, 1993) and rose (Kerssies *et al.*, 1995) flowers and RH have been reported. The effects of equilibrium relative humidity (ERH.) on the *in-vitro* growth of *B. cinerea* were studied by Alam *et al.*, (1996). According to these studies, *B. cinerea* colony diameter continually decreased as a result of reducing ERH from 99.3-94.4%.

2.3.3.3 Rainfall and wetness

Rainfall and wetness of plant surfaces can play an important role in infection initiation (Jarvis 1964). The interruption of a non-aggressive phase of chocolate spot disease caused by *B. fabae* on beans by an aggressive one was related to heavy rainfalls (Wilson, 1937). Substantial factors for leaf specking aggressiveness were high concentrations of *B. fabae* conidia and continuous rain that maintained a water film on the leaves. The promotive effects of surface wetness and rainfall on *B. cinerea* infection on strawberry and raspberry fruits were noted by Jarvis (1964). Free water also possibly helps the transition from a latent infection to the aggressive state of the disease (Jarvis, 1964). A 6-year survey was undertaken to correlate grey mould incidence on strawberry and raspberry crops and rainfall levels in the flowering period 11-20 days before picking.

During the strawberry flowering season, the number of hours over a 5-day period in which relative humidity exceeded 80% was found to have a direct effect on disease incidence (Jarvis, 1964). The effect of wetness duration at a range of temperatures on grape infection by *B. cinerea* has also been examined (Broome *et al.*, 1995). Incidence of berry infection increased with increasing wetness duration at all temperatures tested (12-30°C). Wetness duration also had significant effect on *B. cinerea* infection of strawberries (Bulger *et al.*, 1987). Disease incidence was facilitated with increasing temperatures. Lesions on onions caused by *B. squamosa* appeared 48h after inoculation when dew persisted for 6h and the temperature was 20°C (Shoemaker and Lorbeer, 1977). However, when onion leaves remained wet for 40h, lesions occurred over a wide range of temperatures from 9-23°C.

2.3.3.4 Forecasting

There have been attempts to forecast *Botrytis* disease incidence in the field or in storage on the basis of preharvest meteorological data. Jarvis (1964) suggested that forecasting would be challenging due to complexity of meteorological, edaphic and biotic factors that affect plant-pathogen interaction. Forecasts of epidemics generally utilise preceding weather data over periods not less than 48h ahead (Jarvis, 1977). This period is the normal time for *B. cinerea* infection to occur (Jarvis, 1980b). However, disease forecasts could not determine whether infection will become latent, non-aggressive, or aggressive (Jarvis, 1964).

Monitoring the dispersal of conidia and airborne inoculum population cannot forecast an epidemic if it is not correlated with weather records (Jarvis, 1980b). Airborne *B. cinerea* inoculum and lesion development inside gerbera (Kerssies, 1993) and rose (Kerssies *et al.*, 1995) glasshouses in Holland did not follow a seasonal pattern. Correlation of weather conditions to *B. cinerea* disease incidence on strawberries and raspberries could not be used as a basis for applying protective fungicides to the crops. This is possibly because during the collection of data over a 5-day period, infection by the pathogen could have already occurred (Jarvis, 1964). Herve and Moysan (1967) devised an empirical method of forecasting grey mould on strawberries. According to

their method, the number of hours per day when humidity exceeded 90% was plotted against the average day temperature (°C). An infection period was likely when the curve for mean daily temperature and that for h day^{-1} >90% RH intercept in the numerical zone 14-16 on the y-axis. Three interceptions within 48h indicated the likelihood of an epidemic (Herve and Moysan, 1967). Sutton *et al.* (1986) developed a forecasting system called BOTCAST to assess the best time for fungicide spray applications. This system was based on the relationship between the weather conditions, the sporulation and the infection stages of *B. squamosa* on onions. For BOTCAST, a daily disease index and a cumulative disease index, where disease infection on plants had already occurred, were generated.

2.4 CONVENTIONAL DISEASE CONTROL

2.4.1 Cultural control

Introduction of pathogens into a new area with healthy plants can result in the onset of an epidemic (Agrios, 1997). There are a variety of cultural disease control practices that can help to avoid infection of healthy plant tissues by pathogens. For example, all host plants infected by or suspected of harbouring the pathogens may have to be removed and burned. This practice can help to reduce the spread of the disease to uninfected plants grown in the same area. Moreover, crop residues should be removed from the cultivation area to eliminate survival of the pathogen.

Jarvis (1980b) stated that control of pathogens like *B. cinerea* could be partially accomplished by manipulating the environment to disrupt as many of the developmental stages of the fungus as possible. Decreasing periods of RH in excess of 90% decreased *B. cinerea* incidence (Whinsprear *et al.*, 1970). Similarly, conidial germination can be inhibited by reducing RH and leaf wetness duration (Elad and Shtienberg, 1995). Ventilation of non-heated greenhouses can prevent high RH levels and leaf wetness (Elad and Shtienberg, 1995). Ventilation during winter in greenhouses in Portugal decreased

grey mould incidence as compared to non-ventilated tomato greenhouses (Meneses and Mondeiro, 1990).

Epton and Richmond (1980) have demonstrated the importance of light in sporulation of *B. cinerea.* Reduction of grey mould incidence on cucumber was achieved by using polyvinyl chloride film in greenhouses (Honda *et al.,* 1977). This film absorbs UV-A light screening out light at wavelengths shorter than 390 nm. Films that do not absorb near ultraviolet light have been generally shown to reduce the *in-vitro* production of conidia by several fungi affecting greenhouse crops, such as *B. cinerea* and *Alternaria solani* (Vakalounakis, 1991). In greenhouse experiments in Crete, a long wavelength infra-red absorbing (IRA)-vinyl film reduced grey mould on two tomato cultivars by 40-50% (Vakalounakis, 1991).

2.4.2 Biological control

Filamentous and other fungi isolated from soil or plant parts have been used as antagonists against *Botrytis* diseases (Ippolito *et al.,* 1997; Dik *et al.,* 1999). Fungal and bacterial antagonists have been tested *in-vitro* and *in-planta* by researchers around the world (Table 2.2). This research on microbial antagonists for the control of postharvest diseases including those caused by *B. cinerea* has shown promising results for a range of crops (Ippolito *et al.,* 1997; Lima *et al.,* 1997; Droby *et al.,* 1998) (Table 2.2). Fifty two bacterial isolates were obtained from organically grown strawberry fruits (Moline *et al.,* 1999). From those, 11 were capable of interfering with *B. cinerea* conidial germination. Bacterial isolates showed inhibition of *B. cinerea* growth on agar plates *in-vitro* and reduced grey mould on artificially inoculated harvested strawberry fruit (Moline *et al.,* 1999). *T. harzianum* T39 has been registered in Israel as Trichodex and may be registered in the United States against grey mould of grapes (Wilson 1997). Similarly, *Gliocladium* spp. may be registered for use against grey mould of strawberries.

Table 2.2: Antagonistic activity against various fungal pathogens infecting different of crops.

Host	Pathogen	Antagonist	Reference
Vegetables			
Beans	*B. cinerea*	*Trichoderma viride*, *Alternaria alternata*, *Drechslera* sp., *Myrothecium verrucaria* and *Gliocladium roseum*	Hannusch and Boland, 1996
Bean leaves	*B. cinerea*	*Epicoccum nigrum*, *G. roseum* and *T. harzianum*	Szandala and Backhouse, 2001
Cucumber and tomato	*B. cinerea*	*A. pullulans*, *Cryptococcus luteus*, *C. laurentii*, *C.albidus*, *Gliocladium catenulatum*, *G. roseum* *Trichoderma harzianum*, *T. hamatum*, *T. viride*, *Chaetomium globosum*, *Ulocladium atrum*, *Bacillus pumilus*, *Bacillus* sp., and *Pseudomonas* spp.	Dik *et al.*, 1999
Fruits			
Apple fruit and table grapes	*Botrytis cinerea*, *Penicillium expansum*, *R. stolonifer* and *Aspergilus niger*	*A. pullulans*	Castoria *et al.*, 2001
Grape fruit, table grape and cherry tomatoes	*B. cinerea*, *Penicillium digitatum*, *R. stolonifer* and *A. niger*	*A. pullulans*	Schena *et al.*, 1999

Host	Pathogen	Antagonist	Reference
Strawberry fruit	*B. cinerea*	*Ulocladium atrum*	Boff *et al.*, 2001
Strawberry fruit	*B. cinerea*	*Chryseobacterium indologenes and Pseudomonas putida*	Moline *et al.*, 1999
Strawberry fruit	*B. cinerea*	*A. pullulans*	Adikaram *et al.*, 2002
Strawberry fruit	*B. cinerea* and *R. stolonifer*	*A. pullulans* and *Candida oleophila*	Lima *et al.*, 1997
Tomato	*B. cinerea*	*T. harzianum*	O' Neil *et al.*, 1996

The biopesticide (Bio-Save 100) is based on the bacteria *Pseudomonas syringae* (ESC-11-L-59-66). It is formulated as a 10% wetable powder and is used for protection of apple wounds by various postharvest pathogens (Janisiewicz and Jeffers 1997). Bio-Save 110 is also used for the control of grey mould of pears in storage (Stack, 1998).

2.4.3 Chemical control

There are about 14 most known fungicides for the pre- and postharvest control of *B. cinerea* (Table 2.3). The best-known chemical classes for *B. cinerea* control include the chlorobenzenes, benzimidazoles and dicarboximides (Panagiotarou and Chrisagi, 1998).

Table 2.3: The most important chemical compounds used to control pre- and postharvest infection by *B. cinerea.*

Host	**Treatment**	**Chemical (active ingredient name)**	**Reference**
Geraldton waxflower	Postharvest	Pyrimethanil, iprodione	Taylor *et al.*, 1999
Roses	Postharvest	Prochloraz, iprodione, dichlofluanid,	Elad, 1988
Roses	Postharvest	Picro-cupric-ammonium formate	Hammer and Marois, 1988
Ruscus	Postharvest	Tebuconazole, polyoxin D and polyoxin B	Elad *et al.*, 1993b
Various crops	Preharvest/ postharvest	Sulphur, phtalimids, thiram, chlorothalonil, diethophencard, prochloraz and tebuconazole	Panagiotarou and Chrisagi, 1998
Various crops	Preharvest/ postharvest	Folpet, captan, chlorothalonil dichlofluanid,	Ogawa *et al.*, 1977

2.5 DISEASE GENETICS AND HOST PATHOGEN INTERACTIONS

2.5.1 Gene-for-gene concept

Host-pathogen specificity involves factors that determine the virulence of the pathogen and also factors that confer resistance on the host (Lucas, 1997). Many theories have been proposed concerning mechanisms by which pathogens either achieve or fail to infect host tissue. A model concerning specific gene-for-gene interactions determining the host range of pathogens in wild plant species was first proposed by Flor (1971). In a gene-for-gene system, recognition of the pathogen by the host occurs when a resistance (R) gene of the host interacts with an avirulence (*avr*) gene of the pathogen (Table 2.4).

Table 2.4: Quadratic check of gene combinations and disease reaction types in host-pathogen systems in which the gene-for-gene concept for one gene operates (Lucas, 1997).

Virulent or avirulent Pathogen genes	**Resistant or susceptible genes in the plant**	
	R (resistant) dominant	r (susceptible) recessive
A (avirulent) dominant	AR (-)[a]	Ar (+)
a (virulent) recessive	aR (+)	ar (+)

[a] (-) indicate incompatible interaction and, therefore, no infection. (+) indicate compatible interaction and, therefore, infection.

According to this model, *avr* gene products secreted by hyphae or located on the surface of the pathogen bind to receptor located on the cell membranes of the host epidermal cells. Binding triggers a cascade of defence responses by the host. Every other possible match in the system could lead to infection (Table 2.4). Thus, a combination of a resistant host gene and a virulent pathogen leads to a compatible host-pathogen interaction. Moreover, in both cases when an *avr* race of the pathogen matches

with a susceptible host and a virulent pathogen matches with a susceptible host, the host fails to recognize the pathogen and infection occurs (Flor, 1971).

Culture filtrates or extracts from microbial cells can act as potent inducers of plant defence responses (Chappell and Hahlbrock, 1984; Kombrink and Hahlbrock, 1986; Fritzemeier *et al.,* 1987; Keller *et al.,* 1999). For instance, extracts from fungal cell walls when applied to plant tissue induced the synthesis and accumulation of phytoalexins (Yoshikawa *et al.,* 1993). Active components in such chemical, biological and physical extracts are referred to as elicitors. This term is now generally used to denote agents, which induce plant defence responses, including accumulation of PR-proteins, cell wall structural (strengthening) changes, and hypersensitive cell death (Kombrink and Hahlbrock, 1986).

2.5.2 Structural defence mechanisms

During fungal invasion the pathogen has to overcome preformed structural barriers of the host. Penetration of cell walls can occur both by mechanical pressure applied by fungal infection structures (i.e. appressorium, infection peg) and/or by the secretion of specific enzymes that degrade cell wall components (Mansfield, 1980; Isaac, 1992; Elad, 1997). A thicker cuticle may enhance natural disease resistance by providing increased mechanical resistance (Elad and Everson, 1995). After penetration of preformed structural barriers plants usually respond with the formation of additional resistant structures which may inhibit fungal growth inside host tissue (Mansfield, 1980; Isaac, 1992; Elad, 1997). The most important of the structures are formed by cell walls and by tissues ahead of and around invading hyphae (Mansfield, 1980; Elad, 1997). Callose papillae formed on the inner side of cell walls to prevent growth of invading pathogen are usually produced within 2-3 min after wounding and within 2-3h after artificial inoculation with microorganisms (Agrios, 1997). A lignituber formed as a consequence of extra deposition of cellulose, callose, lignin and other phenolic compounds surrounds the invading hyphae, making further extension through cells more difficult (Elad, 1997).

Histologically observed defence structures are formed after infection by fungi, bacteria and sometimes by viruses and nematodes (Agrios, 1997). These structures include cork layers and tyloses. Cork cells are formed as a result of cambial activity and surround the site of infection (Isaac, 1992). Cork layers inhibit pathogen growth by physically restricting further penetration. However, a perhaps more important function of cork layer cells is the isolation of the invading pathogen from nutrient supplies from inside the cell. The diffusion of pathogenic toxins secreted by the pathogen is also inhibited along with infection of healthy adjacent cells (Isaac, 1992). Abscission layers can be formed on leaves when cork cells surround and isolate the infection site, which becomes a necrotic spot. Necrotic tissue is often separated and may fall out of the leaf causing a "shot" hole. Tyloses are formed inside xylem-vessels and prevent the spread of invading vascular pathogens (Isaac, 1992; Agrios, 1997). Tyloses are a result of protoplast swelling and incursion from adjacent cells. They usually act as a barrier to further expansion of the vascular pathogen inside xylem vessels.

2.5.3 Rapid defence responses

2.5.3.1 The role of reactive oxygen species in plant defence

The first step in the rapid defence responses by plants is recognition of the infection attempt by the pathogen. Pathogen recognition results in a signaling cascade to neighbouring cells and in initial molecular defence responses (Kombrink and Somssich, 1995). Examples of elicitor-active components produced by pathogenic fungi include the β-glucan elicitor and the 42 kDa glycoprotein derived from the fungus *Phytophthora megasperma*, the oligo-1,4-α-galacturonides from *Cladosporium fulvum* and *Rhynchosporium secalis*, and the harpin protein from *Erwinia amylovora.* These compounds activate defence responses when they bind to host receptors during incompatible host-pathogen interactions (Ebel and Cosio, 1994; Kombrink and Somssich, 1995). In parsley cells, the existence of a receptor was proposed by Ebel and Cosio (1994). The intracellular changes were subsequent signals activated by the receptor and

transported to host plasma membrane. Changes in H^+, K^+, Cl^- and Ca^{2+} fluxes across the plasma membrane and H_2O_2 increase within 2-5 min can occur (Nurnberger *et al.,* 1994; Nurnberger and Scheel, 2001).

The activity of active oxygen species (e.g. O^-, H_2O_2) and the rapidity of their production after invasion characterize the rapid defence response of the host (Dixon *et al.,* 1994; Ebel and Cosio, 1994; Bolwell, 1999). These toxic active oxygen species cause host cell death at the infection site (Kombrink and Somssich, 1995). It has been suggested that reactive oxygen species (ROS) could have a dual function in disease resistance (Kombrink and Schmelzer, 2001). Firstly, ROS participate directly in cell death during HR and, thereby, results in direct pathogen inhibition. Secondly, ROS have a role in signal diffusion for cellular protectant induction and associated defence responses in neighbouring cells (Kombrink and Schmelzer, 2001).

2.5.3.2. Hypersensitive response (HR)

The HR is part of the initial plant defence responses and involves localized cell death at the infection site (Kombrink and Schmelzer, 2001). Thus, the HR is a result of host recognition of infection attempts made by a pathogenic bacterium or fungus. Specific elicitors molecules comprise signals, which induce these initial defence responses. When pathogenic bacteria are injected inside a non-host plant under artificial conditions, they are killed by the HR as a result of being surrounded by dead cells. The HR may occur when either virulent strains of bacteria are injected inside a resistant host or avirulent strains of bacteria are injected inside a susceptible host (Agrios, 1997). HR associated isolation of the pathogen inside necrotic cells causes the pathogen loses its ability to take-up nutrients and grow into adjacent healthy cells (Kombrink and Schmelzer, 2001).

Elicitors which do not cause an HR can also activate defence-related compounds (Schroder *et al.,* 1992; Atkinson, 1993; Kuć 1995; Kombrink and Schmelzer, 2001). Activation of these compounds can be similar for both compatible and incompatible host-pathogen interactions (Schroder *et al.,* 1992; Kombrink and Schmelzer, 2001). However, only with compatible interactions does the pathogen infect and colonize the host.

Accumulation of phytoalexins can occur as part of the HR (Dixon *et al.,* 1994). However, it is not clear whether the HR triggers the production of phytoalexins and other antimicrobial compounds or if their accumulation is a direct result of elicitation (Kombrink and Somssich, 1995).

2.5.4 Local acquired resistance

2.5.4.1 Phytoalexins

Phytoalexins are low molecular weight antimicrobial compounds produced *de-novo* by some plants. They accumulate during infection by pathogens or after injury or stress (Ebel, 1986; Isaac, 1992; Kuć, 1995). Accumulation of phytoalexins is mainly observed when fungi, rather than bacteria, viruses or nematodes, try to infect the plant. Accumulation is a result of specific elicitors released either by the fungal cell walls or by the plant cell walls (Ebel, 1986). Elicitors of phytoalexins include a large number of compounds including inorganic salts (Perrin and Cruickshank, 1965), oligoglucans (Sharp *et al.,* 1984), ethylene (Chalutz and Stahmann, 1969), fatty acids (Bostock *et al.,* 1981), and chitosan oligomers (Kendra and Hadwiger, 1984). Over 200 compounds, microorganisms and physiological stresses have been reported to elicit pisatin in pea, phaseollin and kievitone in green bean and glyceollin in soybean (Kuć, 1991).

Most phytoalexins have been isolated from dicot plants, but they are also present in monocots including rice, corn, sorghum, wheat, barley and onions (Kuć, 1995). There is no published work on phytoalexins in cut flower species. Phytoalexins have been found in almost every part of the plant including roots, stems, leaves and fruits (Kuć, 1995). Such plant species produces a characteristic set of phytoalexins derived from secondary metabolism, in most cases via the phenylpropanoid pathway (Ebel, 1986; Kombrink and Somssich, 1995; Kuć, 1995). Phytoalexins belong to a number of key chemical groups including phenolics (e.g. flavonoids and coumarins), polyacetylenes, isoprenes, terpenoids and steroids (Ebel, 1986). They are produced by both resistant and susceptible tissues and resistance appears to be related with the total phytoalexin

concentration (Kuć, 1995). Phytoalexins can affect fungal growth by inhibiting germ tube elongation and colony growth (Elad, 1997). The main effect of phytoalexins on fungi is via their cell membranes. Direct contact of phytoalexins with fungal cell walls resulted in fungal plasma membrane disruption and loss of the ultrastructural integrity (Elad, 1997). In compatible interactions, the pathogen apparently tolerates accumulated phytoalexins, detoxifies them, suppresses phytoalexin accumulation and/or avoids eliciting phytoalexin production (Kuć, 1995). Overcoming phytoalexin accumulation is attributed to either suppressor molecules released by the pathogen (i.e. low molecular weight polysaccharides or glycopeptides) or suppression of the intensity and timing of signal genes that could trigger phytoalexin accumulation (Kuć, 1995).

2.5.4.2 Pathogenesis related proteins (PR-proteins)

Pathogenesis related proteins (PR-proteins) accumulate either in extracellular space or the vacuole after various types of plant stress, including pathogen infection (Stermer, 1995; Sticher *et al.*, 1997). PR-proteins accumulate at the site of infection as well as in uninfected tissues (Van Loon and Gerritsen, 1989; Ryals *et al.*, 1996). Although healthy plants may contain traces of PR-proteins, the transcription of genes encoding PR-proteins is up-regulated following pathogen attack, elicitor treatment, wounding or stress (Stermer, 1995; Sticher *et al.*, 1997; Van Loon, 1997). Signal compounds responsible for initiating PR-protein production include salicylic acid, ethylene, the enzyme xylanase, the polypeptide systemin and jasmonic acid (Agrios, 1997). The importance of PR-proteins lies in their range of defence activities (Van Loon *et al.*, 1994). A number of PR-proteins release molecules that may act as elicitors (Keen and Yoshikawa, 1983). PR-proteins accumulation has been observed in monocots as well as in dicots (Redolfi, 1983). However, there is no published work on PR-proteins induced in flower species. Eleven families of PR-proteins have been recognized so far (Van Loon *et al.*, 1994). Some inhibit pathogen development during microbial infection by inhibiting fungal spore production and germination. Others are associated with strengthening of the host cell wall via its outgrowths and papillae (Agrios, 1997). Both β-1,3-glucanases and chitinases, PR-2 and PR-3, respectively, are known to have direct

antifungal activity (Mauch *et al.,* 1988; Van Loon, 1997). However, many pathogens have evolved mechanisms to reduce the antifungal impact of PR-proteins (Van Loon, 1997). For example, many chitin-containing fungi are not inhibited by host-produced chitinases.

PR-proteins are also called systemic acquired resistance (SAR) proteins due to their accumulation during the expression of SAR (Ward *et al.,* 1991; Kessmann *et al.,* 1994; Sticher *et al.,* 1997). They can have relative species specificity. For example, PR-1 was found in tobacco and *Arabidopsis* during SAR expression. In contrast, in cucumber, extracellular chitinase PR-3 was highly expressed rather than PR-1 expression (Kessmann *et al.,* 1994).

2.5.4.3 Secondary metabolites and the role of phenylalanine ammonia-lyase (PAL)

Plant secondary metabolites are divided into the three main categories of terpenes, phenolic compounds and nitrogen containing secondary metabolites (i.e. alkaloids) (Taiz and Zeiger, 1998). All secondary metabolites are produced through one of the major mevalonic, malonic or shikimic pathways (Taiz and Zeiger, 1998). Phenylalanine is a common amino acid produced via the shikimic pathway (Hahlbrock and Scheel, 1989) (Figure 2.2). The most abundant classes of secondary phenolic compounds in plants are derived from phenylalanine via elimination of an ammonia molecule to form cinnamic acid. This reaction is catalyzed by phenylalanine ammonia lyase (PAL), the key enzyme of phenylpropanoid metabolism (Hahlbrock and Scheel, 1989). Derivatives of phenylpropanoid pathway include low-molecular-weight flower pigments, antibiotic phytoalexins, UV-protectants, insect repellents, and signal molecules in plant-microbe interactions (Hahlbrock and Scheel, 1989; Kombrink and Somssich, 1995).

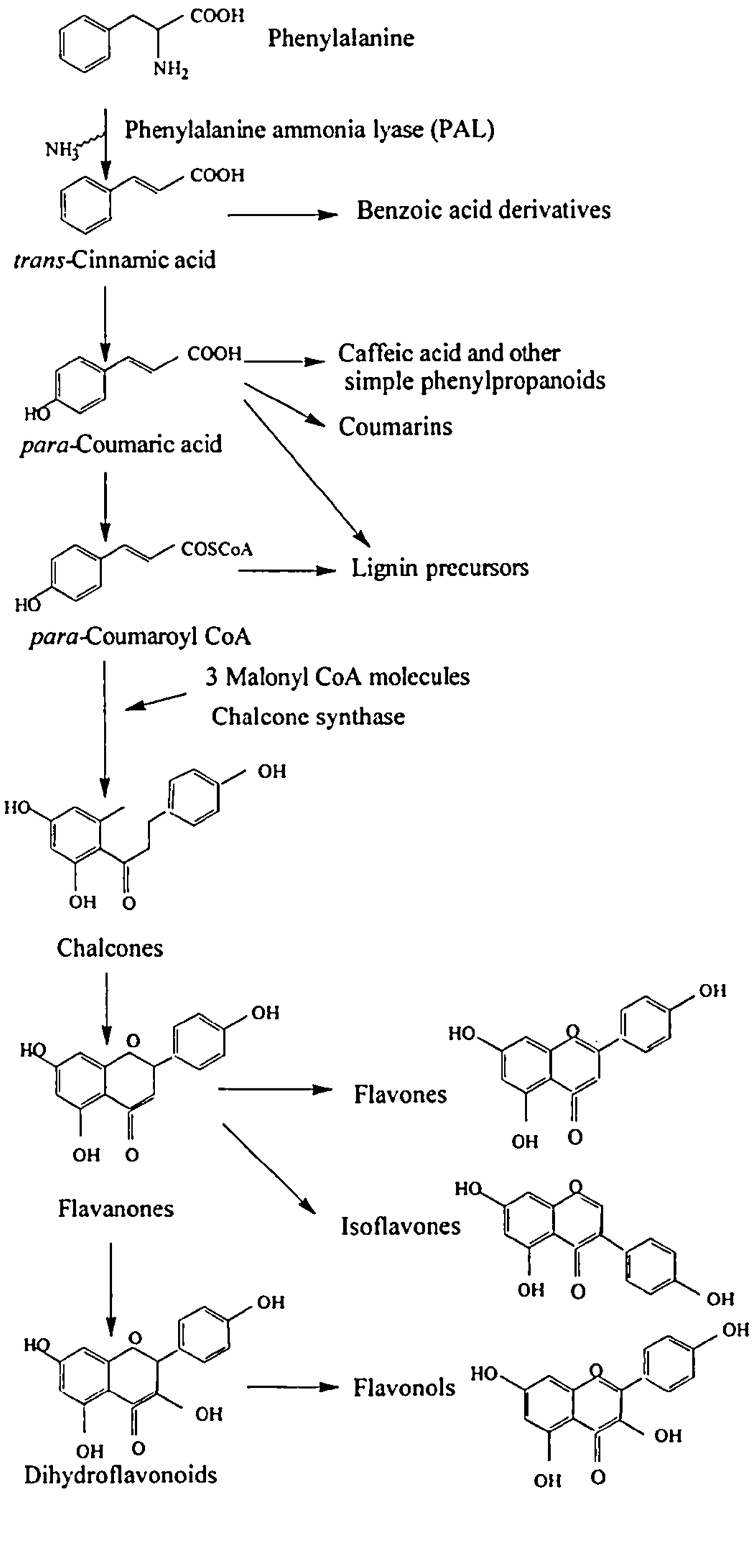

Figure 2.2: Phenylpropanoid biosynthesis from phenylalanine. Formation of plant phenolics, coumarins, benzoic acid derivatives, lignin, anthocyanins, isoflavones, condensed tannins and other flavonoids begins with phenylalanine.

The main phenylpropanoid pathway branches leading to formation of flavonoids, isoflavonoids, coumarins, soluble esters, wall bound phenolics, lignin and suberin. This diverse spectrum of compounds has a wide range of properties (Hahlbrock and Scheel, 1989). For example, the lignin pathway is an important phenylpropanoid pathway branch that produces precursors for lignin deposition (Grisebach, 1981). Various enzymes implicated in the biosynthesis of lignin appeared to be induced in plants in response to infection or elicitor treatment (Matern and Kneusel, 1988). However, not all studies show a role of lignin and cell lignification in disease inhibition (Ride and Pearce, 1979; Garrod *et al.*, 1982). Furanocoumarins derived from the furanocoumarin pathway in parsley are considered potent phytoalexins (Beier and Oertli, 1983). Flavonoid and furanocoumarin production as a response to UV light or fungal elicitor treatment respectively was associated with up-regulation of PAL, 4-coumarate: CoA-ligase (4CL) and chalcone synthase (CHS). Up-regulation was based on rapid changes in amounts and activities of the corresponding mRNAs (Chappell and Hahlbrock, 1984). Increased PAL activity was observed when potato tubers were inoculated with the fungus *Phytophthora infestans* during either a compatible or incompatible host-pathogen interaction (Hahlbrock and Scheel, 1989). Artificial inoculation of potato leaves with *P. infestans* resulted in rapid stimulation of phenylpropanoid metabolism (Fritzemeier *et al.*, 1987). Increases in PAL and 4CL mRNAs were detected within a few hours after inoculation. Similar changes in PAL and 4CL mRNAs were recorded in resistant potato cultivars inoculated with either virulent or avirulent fungal strains (Fritzemeier *et al.*, 1987). The importance of PAL in the local defence responses of barley was demonstrated by Carver *et al.* (1994). Barley plants were treated with the PAL inhibitors AOA (α-aminooxy acetic acid) and AOPP (α-aminooxy-β-phenylpropionic acid) and then they were artificially inoculated with the fungus *Erysiphe graminis* f.sp. *avenae*. Appressorium and haustorium formation were greater for plants treated with the PAL inhibitors than for untreated control plants. Mauch-Mani and Slusarenko (1996) showed that when *Arabidopsis* plants were treated with the PAL inhibitor 2-aminoindan-2-phosphonic acid (AIP), the incompatible interaction with an avirulent strain of the fungus *Peronospora parasitica* became a fully compatible one. Total colonization of the plants by the fungus was associated with reduction of CAD (cinnamyl-alcohol dehydrogenase) activity which

produces a lignin precursor. Suppression of salicylic acid concentrations in plants treated with AIP was also observed (Mauch-Mani and Slusarenko, 1996). Cultured parsley cells (*Petroselinum crispum*) responded to elicitor treatment from different phytopathogenic fungi by forming coumarin derivative phytoalexins (Kombrink and Hahlbrock, 1986). Induction of coumarins occurred via marked but transient increases in PAL and 4CL activities.

2.5.4.4 Salicylic acid (SA)

After pathogen recognition by the host, a cascade of early responses is induced including ion fluxes, phosphorylation events, and generation of active oxygen species (Kombrink and Somssich, 1995). SA acts as a secondary signal molecule and its levels increase during the defence induction process. Thus, SA is required for initiation of synthesis of various defence-related proteins such as the PR-proteins (Gaffney *et al.*, 1993; Van Loon, 1997; Metraux, 2001). SA accumulation endogenously in tobacco and cucumber plants lead to the HR and the SAR responses. However, SA is not necessarily the translocated signal (elicitor) for the onset of SAR. Rather, SA exerts an effect locally (Vernooij *et al.*, 1994; Ryals *et al.*, 1996). Nonetheless, SA is still required for SAR expression (Van Loon, 1997). The importance of SA in the onset of SAR was determined using transgenic tobacco and *Arabidopsis* plants engineered to over-express SA-hydroxylase. This enzyme, from *Pseudomonas putida*, is involved in naphthalene metabolism and catalyzes the conversion of SA to catechol (Gaffney *et al.*, 1993; Delaney *et al.*, 1994). Transformed plants with the naphthalene hydroxylase G (*NahG*) gene produced low levels of SA and, therefore, SAR expression was blocked. The requirement of SA for SAR expression was clearly demonstrated.

SA is produced from phenylalanine via coumaric and benzoic acid (Mauch-Mani and Slusarenko, 1996; Ryals *et al.*, 1996; Sticher *et al.*, 1997). Biosynthesis of SA starts with the conversion of phenylalanine to *trans*-cinnamic acid (Sticher *et al.*, 1997). From *trans*-cinnamic acid, either benzoic acid (BA) or *ortho*-coumaric acid are produced. Both compounds are SA precursors (Sticher *et al.*, 1997). Pallas *et al.* (1996) showed that tobacco plants epigenetically suppressed in PAL expression produced a much lower

concentration of SA and other phenylpropanoid derivatives when artificially inoculated with tobacco mosaic virus (TMV). This was seen, firstly, due to the lack of resistance to TMV upon secondary infection, and, secondly, to the absence of PR protein induction in systemic leaves (Pallas *et al.*, 1996).

2.5.4.5 Jasmonic acid (JA)

Jasmonic acid (JA) and its methyl ester (MeJA) are derived from linolenic acid (Figure 2.3). They are cyclopentanine-based compounds that occur naturally in many plant species (Sembdner and Parthier, 1993; Creelman and Mullet, 1997).

Linolenic acid levels or its availability could determine JA biosynthetic rate (Farmer and Ryan, 1992; Conconi *et al.*, 1996). The level of JA in plants varies as a function of tissue and cell type, developmental stage, and in response to various environmental stimuli (Creelman and Mullet, 1997). For example, in soybean seedlings, JA levels are higher in the hypocotyls hook (a zone of cell division) and young plumules as compared to the zone of cell elongation and more mature regions of the stem, older leaves and roots (Creelman and Mullet, 1997). High JA levels are also found in flowers and pericarp tissues of developing reproductive structures (Creelman and Mullet, 1997). Jasmonates are wide spread in Angiosperms, Gymnosperms and algae (Parthier, 1991). They can mediate gene expression in response to various environmental and developmental processes (Wasternack and Parthier, 1997). These processes include wounding (Schaller and Ryan, 1995), pathogen attack (Epple *et al.*, 1997), fungal elicitation (Nojiri *et al.*, 1996), touch (Sharkey, 1996), nitrogen storage (Staswick, 1990), and cell wall strengthening (Creelman *et al.*, 1992).

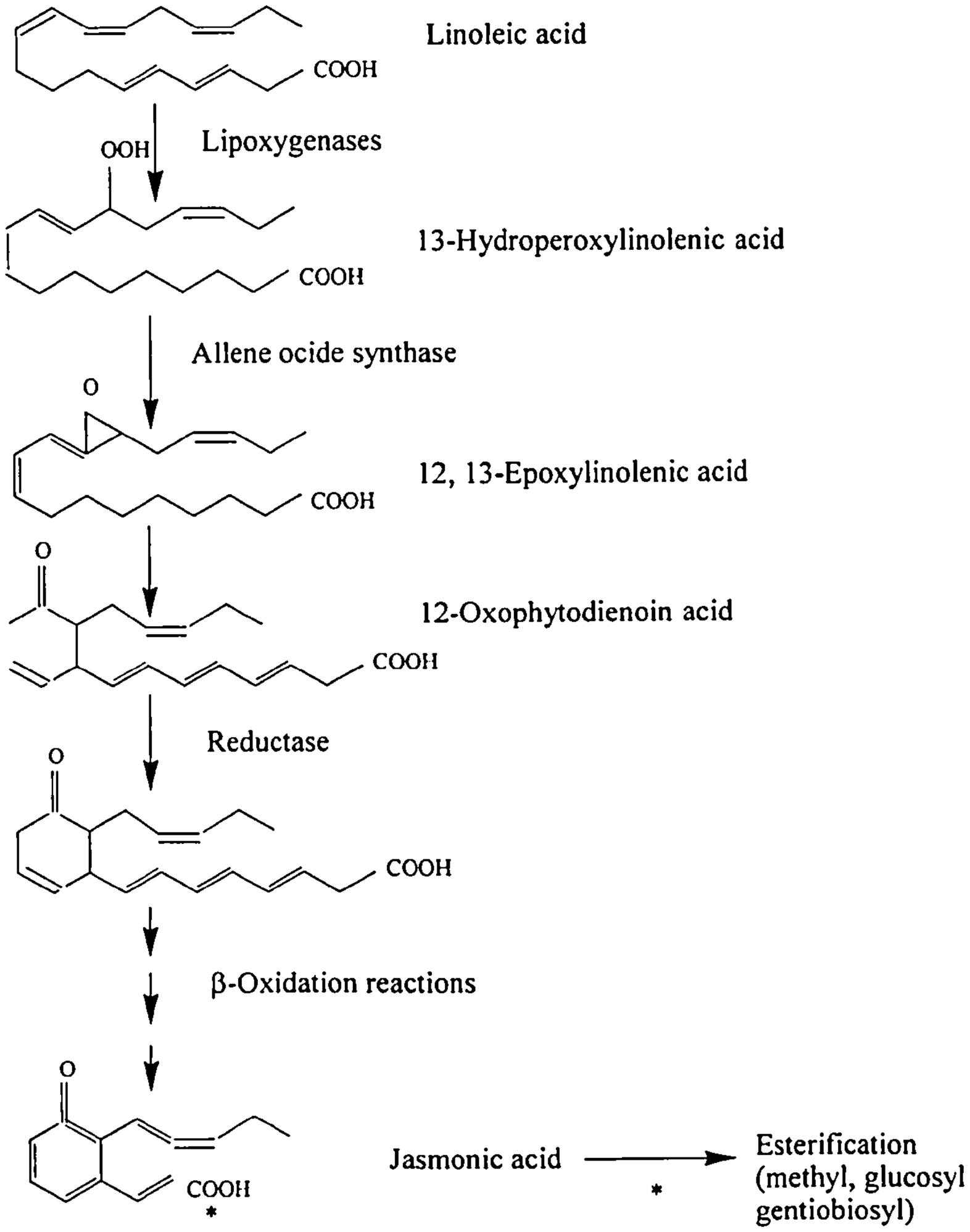

Figure 2.3: Pathway for conversion of linolenic acid to jasmonic acid. Methyl jasmonate is produced after esterification of the carboxyl group (Sembdner and Parthier, 1993).

Wounding of tomato leaves produced an 18-amino acid polypeptide called systemin, the first polypeptide hormone discovered in plants so far (Pearce *et al.*, 1991). Systemin was released from damaged cells into the apoplast and transported out of the wounded leaf via the phloem (Schaller and Ryan, 1995) (Figure 2.4). Systemin was believed to bind to the plasma membrane of target cells and thereby initiate JA biosynthesis (Schaller and Ryan, 1995). JA accumulation can also be induced by oligosaccharides derived from plant cell walls and by elicitors, such as chitosans derived

from fungal cell walls (Gundlach *et al.*, 1992; Doares *et al.*, 1995; Nojiri *et al.*, 1996) (Figure 2.3).

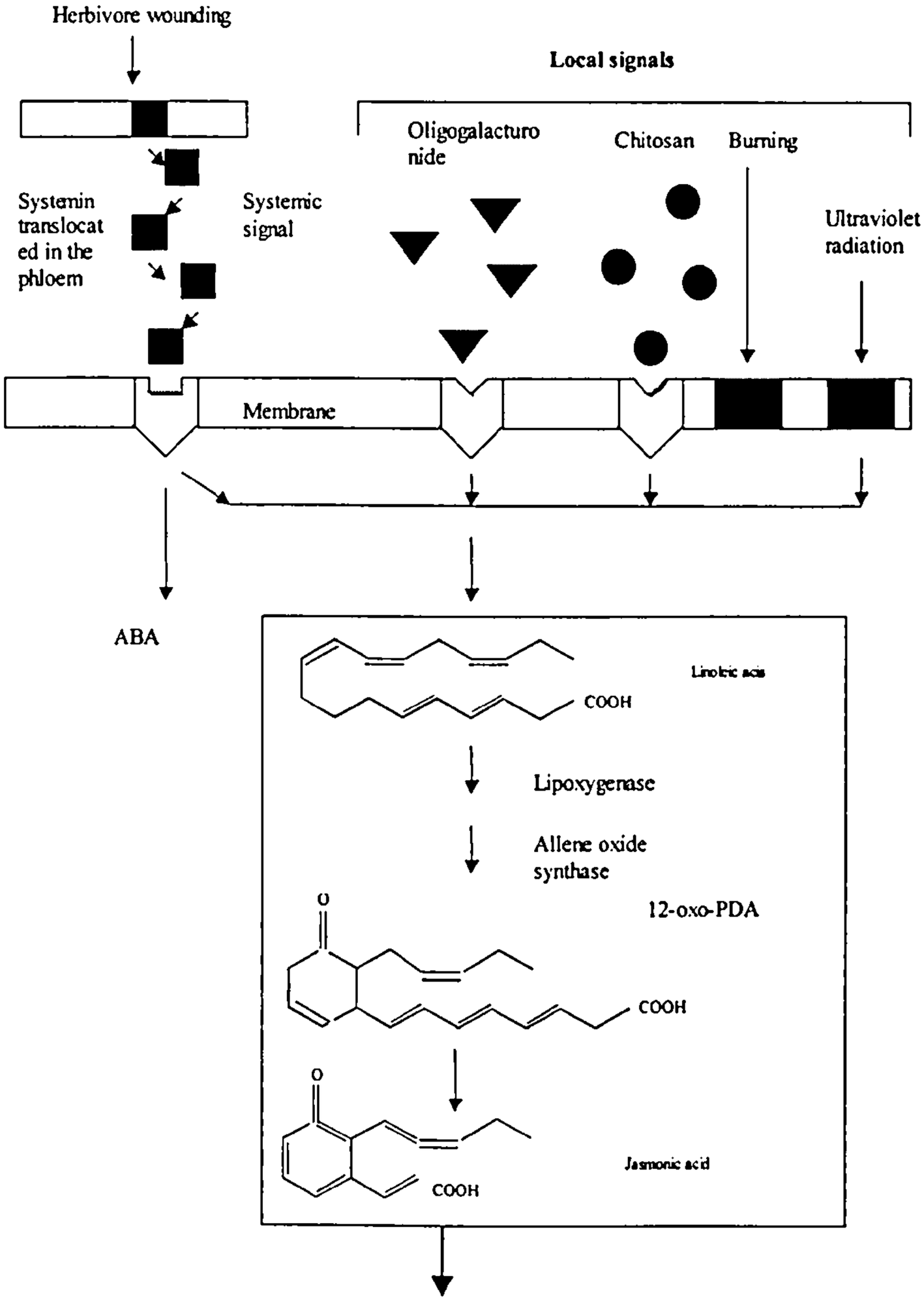

Figure 2.4: The octadecanoid-signaling pathway for defence gene expression in tomato (Schaller and Ryan, 1995). Upon herbivore wounding, a systemic signal is delivered from systemin and results in an ABA-dependent rise of linoleic acid. From linoleic acid, jasmonic acid is produced (Figure 2.2). Ethylene is required in the jasmonic-signaling cascade (O'Donnell *et al.*, 1995).

JA also activates gene expression encoding proteinase inhibitors (Creelman and Mullet, 1997). Proteinase inhibitors are known antidigestive proteins that block the action of herbivore proteolytic enzymes (Creelman and Mullet, 1997). Thereby, proteinase inhibitors help the host to avoid consumption by herbivores. Proteinase inhibitors were accumulated in tomato plants after wounding (O'Donnell *et al.*, 1996) and after irradiation with UV-C (Conconi *et al.* 1996). In response to wounding, ethylene and JA act together to regulate gene expression of proteinase inhibitors (O'Donnell *et al.*, 1996). Exposing tomato leaves to increasing doses of 254 nm UV-C resulted in increased proteinase inhibitors gene expression. Expression of proteinase inhibitors in wounded (Doares *et al.*, 1995; O'Donnell *et al.*, 1996) or UV-C treated (Conconi *et al.*, 1996) tomato leaves was markedly reduced upon treatment with SA.

Lipoxygenase (LOX) is a dioxygenase that is widely distributed in higher plants (Heldt, 1997) (Figure 2.2). LOX catalyses the dioxygenation of multiple unsaturated fatty acids including linolenic and linoleic acids (Heldt, 1997). Treatment with LOX inhibitors reduced the ability of tomato leaves to synthesize JA (Pena-Cortes *et al.*, 1993). While LOX mediates a step in JA biosynthesis its exact role in JA biosynthesis is not yet determined due to the high number of genes encoding for LOX (Park and Polacco, 1989). Exogenous application of both JA and ethylene induced a set of defence genes in *Arabidopsis* (Epple *et al.*, 1997) and radish seeds (Terras *et al.*, 1995) that were also activated upon pathogen infection. These were genes encoding plant defensins and thionins, which are small cysteine-rich basic proteins with antimicrobial activity.

2.5.5 Systemic acquired resistance (SAR)

The first definition of systemic acquired resistance (SAR) was reported by Ross (1961). The tips of tobacco leaves were inoculated with tobacco mosaic virus (TMV) and re-inoculated after a period of time with the same virus. Disease severity and lesion diameter was recorded and compared with tobacco leaves inoculated only once with TMV. Ross (1961) observed that the pre-inoculated leaves expressed a distinct resistance

after the second inoculation and defined the tobacco plants defence activity as systemic acquired resistance (SAR).

SAR is activated following induction of local acquired resistance (LAR). SAR is potentially induced after the HR and after challenge with virulent strains of a pathogen or elicitor treatment. It develops systemically in distant parts of the infected plant (Lawton *et al.*, 1996; Ryals *et al.*, 1996; Metraux, 2001). SAR protects plants from a broad range of potential pathogens (Kessmann *et al,* 1994).

Specific genes induced in different plant species during SAR have been called SAR-genes (Kessmann *et al.*, 1994; Stermer, 1995; Ryals *et al.*, 1996; Sticher *et al.*, 1997). Most of SAR-genes encode PR-proteins such as those accumulated after inoculation of tobacco with TMV (Ward *et al.*, 1991). These include PR-1 (PR-1a, PR-1b, PR-1c), β-1,3-glucanases (PR-2a, PR-2b, PR-2c), chitinases (PR-3a, PR-3b), hevein-like proteins (PR-4a, PR-4b), thaumatin like proteins (PR-5a, PR-5b), acidic and basic isoforms of class III chitinase, an extracellular β-1,3-glucanase and the basic isoform of PR-1 (Ward *et al.*, 1991). SAR and SAR-gene activation has been observed in various dicots (Kessmann *et al.*, 1994; Ryals *et al.*, 1996). SAR activation involves species specificity (Ryals *et al.*, 1992). For example, acidic PR-1 is only weakly expressed in cucumber. In contrast, acidic PR-1 is the main protein accumulating in tobacco and *Arabidopsis.* A number of homologous SAR-genes have been identified in monocots. Homologs of the PR-1 family were found in maize and barley and other PR-proteins in maize (Nasser *et al.*, 1988). Gorlach *et al.* (1996) isolated a group of wheat genes (WCI or wheat chemically induced) induced after chemical treatment with potent SAR inducers. WCI genes seemed to act in a similar manner to SAR-genes in dicots after chemical treatment with plant activators (Gorlach *et al.*, 1996).

The mechanisms of SAR expression in distant tissue areas away from the infection site, as well as the triggering signal for SAR expression, have not been clarified yet. However, SA was present at high concentrations in the phloem of plants during SAR expression (Ryals *et al.,* 1996; Sticher *et al.,* 1997).

2.5.6 Induced systemic resistance (ISR)

Induced systemic resistance (ISR) was first introduced by Chester in 1933. Recent extended work established ISR as a distinct phenomenon occurring in higher plants. Kuć (2000) defined ISR as "the phenomenon whereby resistance to infectious disease is systemically induced by localized infection or treatment with microbial components or products or by a diverse group of structurally unrelated inorganic or organic compounds". ISR can be subdivided into two broad categories (Hammerschmidt, 1999). The first category is SAR as firstly described by Chester in 1933 and then by Ross in 1961. The second category is characterized by a phenomenon described by Pieterse *et al.* (1996). They studied the effects of *Pseudomonas fluorescens* strain WCS417r colonizing *Arabidopsis* roots. Colonized plants were more resistant to infection by *Fusarium oxysporum* f. sp. *raphani* and *P. syringae* pv. *tomato*. This rhizobacteria-mediated ISR response was independent of the SA accumulation and PR gene activation as typically observed during SAR. Using the jasmonate response mutant *jar1* (JA resistant 1), the ethylene response mutant *etr1* (ethylene resistant 1) and the SAR regulatory mutant *npr1* (PR-1 non-expressor), it was demonstrated that signal transduction leading to *P. fluorescens* WCS417r-mediated ISR required JA and ethylene and was dependent on the NPR1 (PR-1 non-expressor) gene (Figure 2.4, pathway 1). It was found that, ISR and SAR pathways were different and their regulation was depended on different elicited signals (Pieterse *et al.*, 1996).

2.5.7 Systemic signaling pathways

Recent research has revealed that JA and ethylene play key roles in signal transduction pathways associated with plant defence responses (Pieterse and van Loon, 1999; Thomma *et al.*, 2000). Inoculation with a necrotizing pathogen resulted predominantly in activation of the SA-dependent SAR response. This response leads to the accumulation of salicylic acid inducible PR-proteins and the expression of SAR (Ryals *et al.*, 1996; Pieterse and van Loon, 1999) (Figure 2.5, pathway 2). JA and ethylene inducible defence responses are induced by non-necrotizing rhizobacteria and

lead to the ISR phenomenon (Pieterse *et al.*, 1996; Pieterse *et al.*, 1998) (Figure 2.5, pathway 1). Both pathways 1 and 2 are regulated in *Arabidopsis* plants carrying the NPR1 gene.

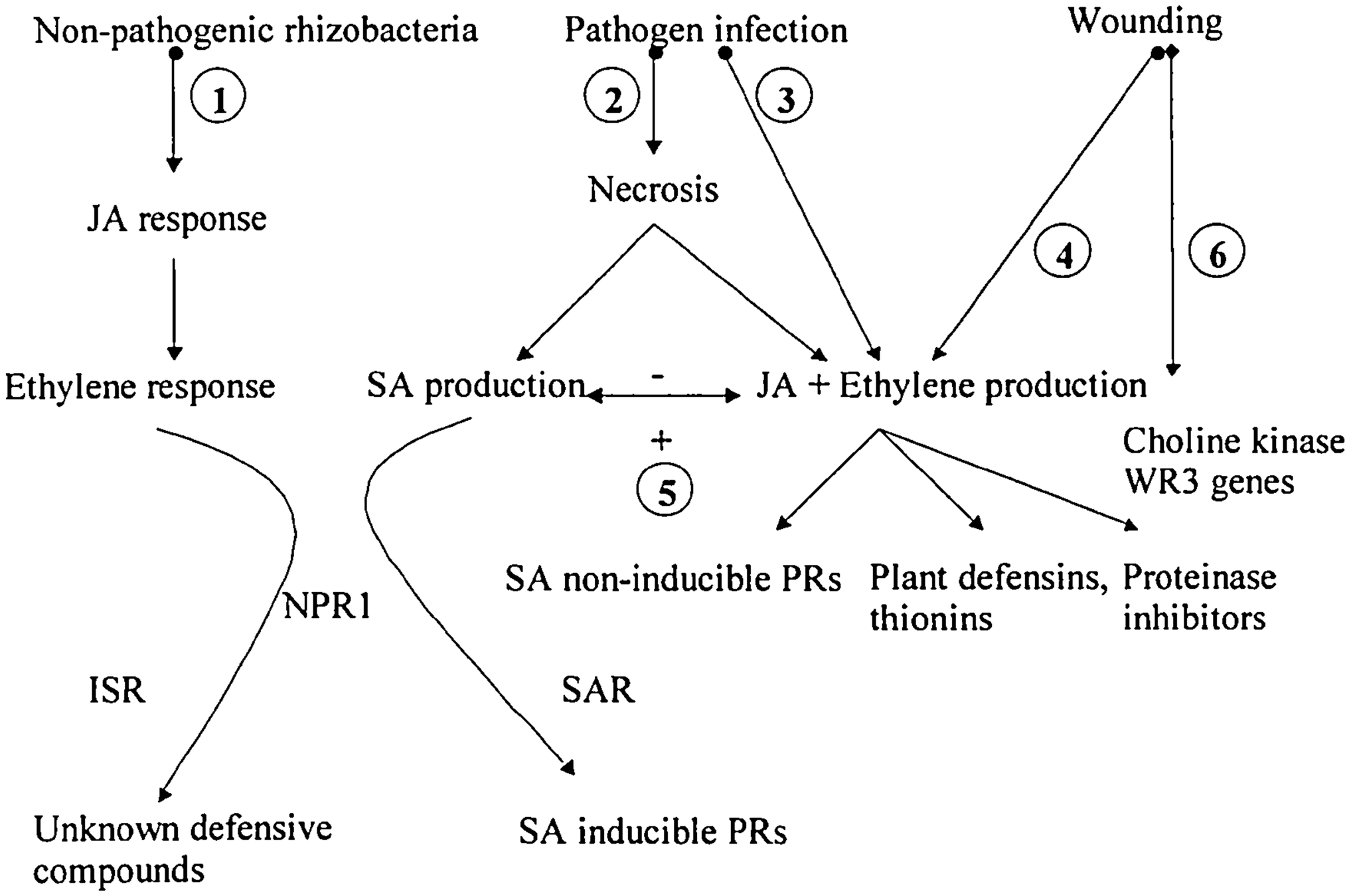

Figure 2.5: Model showing systemic signaling pathways that can be induced in plants by non-pathogenic rhizobacteria, pathogen infection and wounding, such as caused by foraging insects. 1: ISR is induced in NPR1 *Arabidopsis* plants as a result of JA and ethylene responses. 2: SAR is induced in NPR1 *Arabidopsis* plants after necrosis by pathogenic fungi, bacteria or virus. 3: JA/ethylene pathway is up- regulated after fungal infection. JA/ethylene expression leads to genes encoding plant defensins, thionins, proteinase inhibitors and SA-independent PR-proteins. 4 and 6: A number of genes are regulated after mechanical wounding. JA and ethylene levels rise after mechanical wounding. 5: Cross-talk between SA- and JA- dependent pathways exist. Adapted from Pieterse and van Loon (1999).

Depending on the invading pathogen, the composition of defence compounds produced after infection can vary between SA- and JA/ethylene-inducible pathways (Figure 2.5, pathways 2 and 3) (Ryals *et al.*, 1996; Epple *et al.*, 1997; Dong, 1998) (see sections 2.3.2.5. and 2.3.2.6.).

Wounding can also result in JA and ethylene inducible defence response activation (Figure 2.5, pathway 4) (O'Donnell *et al.*, 1996; Wasternack and Parthier, 1997). However, resultant products of the wounding pathway differ from those induced upon pathogen infection (O'Donnell *et al.*, 1996; Rojo *et al.*, 1999). A second distinct wound-signaling pathway leading to wound responsive (*WR*) gene expression has been found in *Arabidopsis* plants (Titarenko *et al.*, 1997; Rojo *et al.*, 1998) (Figure 2.5, pathway 6). Upon wounding, *Arabidopsis* plants carrying the *coi1* (JA-insensitive) mutant gene expressed the wound responsive genes choline kinase (*CK*) and wound responsive (*WR3*) indicating that the induced pathway was totally independent of JA. UV irradiation of tomato leaves also resulted in induction of the same defensive genes normally activated through the octadecanoid pathway after wounding (Conconi *et al.*, 1996). This response is blocked after SA treatment, confirming the antagonistic regulation of the two distinct pathways (Pena-Cortes *et al.*, 1993; Lawton *et al.*, 1995; Xu *et al.*, 1994; Doares *et al.*, 1995; O'Donnell *et al.*, 1996; Niki *et al.*, 1998; Gupta *et al.*, 2000; Rao *et al.*, 2000).

In the rhizobacteria-mediated induced systemic resistance (ISR) pathway, components from the JA/ethylene response acted in sequence in activating a systemic resistance response that, like pathogen induced SAR, was dependent on the regulatory protein NPR1 (Pieterse and van Loon, 1999). The ISR pathway shares signaling events with pathways initiated upon pathogen infection, but is not associated with the activation of genes encoding plant defensins, thionins or PR-proteins (Pieterse and van Loon, 1999) (Figure 2.5, pathway 3). This observation indicates that ISR inducing rhizobacteria, such as *P. fluorescens* strain WCS417r, trigger a novel signaling pathway leading to the production of so far unidentified defense compounds (Pieterse *et al.*, 1996; Pietrese *et al.*, 1998). Protection of *Nah*G *Arabidopsis* plants by gaseous MeJA suggested that induction of a SA non-dependent systemic pathway was regulated by JA (Thomma *et al.*, 2000) (Figure 2.5, pathway 3). Protection was provided against two necrotrophic fungi, *A. brassicicola* and *B. cinerea* (section 2.5.7.2).

Unlike wounding, the ISR response seems to be associated with an increase in sensitivity to JA and/or ethylene rather than to an increase in their production. This subtle difference might led to the activation of different sets of defence genes. Whether some of these unknown ISR defence compounds are also produced upon pathogen attack or wounding needs further investigation (Pieterse and van Loon, 1999).

2.5.7.1 SA and JA/Ethylene dependent pathways cross talk

There is complex cross talk between SA and JA/ethylene pathways (Glazebrook, 2001) (Figure 2.5, pathway 5). However, in most cases up-regulation of one pathway will down-regulate the other (Pena-Cortes *et al.*, 1993; Lawton *et al.*, 1995; Xu *et al.*, 1994; Conconi *et al.*, 1996; Niki *et al.*, 1998; Gupta *et al.*, 2000; Rao *et al.*, 2000). In a recent study by Rao *et al.* (2000), *nahG* plants showed increased tolerance to ozone and decreased SA levels when treated with JA. Mutants carrying the *jar1* and *fad3* (*fatty-acid deficient* 3) genes resulted in decreased ozone tolerance. Expression of the JA-depended gene *PDF1.2* was strongly inhibited by SA (Gupta *et al.*, 2000). SA and JA had opposite effects on the expression of acidic and basic PR genes in mature tobacco leaf discs (Niki *et al.*, 1998). JA worked as a basic PR gene inducer and as an inhibitor of acidic PR genes. On the other hand, SA worked as an acidic PR gene inducer inhibiting the up-regulation of the basic PR genes (Niki *et al.*, 1998). However, synergistic cross talk between salicylic acid (SA) and JA/ethylene dependent pathways could provide regulatory potential for activating multiple resistance mechanisms in plants (Pieterse and van Loon, 1999). This prospect awaits future research.

2.5.7.2 SA and JA/Ethylene are induced by different pathogens

Plants deficient in the SA-dependent defence response (e.g. *npr1* and *NahG*) do not show enhanced susceptibility towards *B. cinerea* indicating that SA was not essential for inhibiting this specific fungus (Thomma *et al.*, 1998; Govrin and Levine, 2002). On the other hand, plants with a deficiency in the JA/ethylene-dependent defence response

[*coi1* (jasmonic acid insensitive) and *ein2* (ethylene insensitive)] displayed enhanced susceptibility to *B. cinerea*, indicating once again that JA/ethylene have a significant role in *B. cinerea* inhibition (Thomma *et al.*, 1998; Govrin and Levine, 2002). SAR response in *Arabidopsis* plants was induced either by artificial inoculation with avirulent *P. syringae* or by being sprayed with SA or acibenzolar (Govrin and Levine, 2002). These plants were then inoculated with *B. cinerea*, and disease severity and lesion diameter were recorded. Lesions produced by *B. cinerea* on induced plants were similar to controls, indicating that SAR induction did not provide any protection against *B. cinerea*. However, the induced plants were resistant to virulent strains of *P. syringae* (Govrin and Levine, 2002). In the same study, *B. cinerea* infecting *Arabidopsis* plants resulted in *PR-1, PR-5, PDF1.2* and *GST1* gene expression. The expression of the *PR-1* gene was also observed in *NahG* plants indicating that induction was SA-independent (Govrin and Levine, 2002).

An evolutionary perspective on SA and JA/ethylene dependent pathways regulation was recently reviewed (Pieterse and van Loon, 1999; Thomma *et al.*, 2001). The SA-dependent pathway is responsive to infection by biotrophic bacteria (i.e. *Pseudomonas*) and fungi (e.g. *Peronospora parasitica* and *Erysiphe orontii*). Resistance to this type of pathogens is often associated with an HR response. In contrast, the JA/ethylene dependent pathway responds to infection by necrotrophic bacteria (e.g. *Erwinia carotovora*) and fungi (e.g. *B. cinerea, Alternaria brassicicola* and *Pythium sp.*). Although the HR can confer protection against biotrophs, it is likely to facilitate the growth of necrotrophs (Thomma *et al.*, 2001).

2.6 ELICITATION OF DEFENCE RESPONSES

Disease management in the past has been achieved by various methods including resistant cultivars, biological control, crop rotation, tillage, and chemical pesticides (Kessmann *et al.*, 1994). Recently, the use of abiotic/biotic agents and synthetic compounds that induce host immune systems have offered a new prospect for disease management.

2.6.1 Biological agents

The cellulase enzyme derived from *Trichoderma viride,* induced a hypersensitive response when applied to a grapevine cell suspension culture (Calderon *et al.,* 1993). Calderon *et al.* (1993) found that this elicitor treatment induced phenolic metabolism, formation of H_2O_2 and synthesis of the stilbene phytoalexin resveratrol. The formation of extracellular peroxidases and a new form of basic peroxidase were correlated with each other and with the formation of resveratrol oxidation products and tissue browning (Carderon *et al.,* 1994). The authors considered this a potential mechanism of induced resistance for future biological control of *B. cinerea.* Zimand *et al.* (1996) found that germination and germ-tube elongation of *Botrytis* conidia was inhibited on bean leaves by *T. harzianum.* Treatment of the green strawberry fruit with *Aureobasidium pullulans* (20×10^5 cells mL^{-1}) appeared to suppress *B. cinerea* by inducing natural disease resistance (Adikaram *et al.,* 2002). It was suggested that the suppression was caused by phytoalexin accumulation stimulate by *A. pullulans*. Extracellular exo-chitinase and β-1,3-glucanase activities were detected both *in-vitro* on agar plates and in apple wounds treated with 30 mL of *A. pullulans* (1×10^8 cells mL^{-1}) (Castoria *et al.,* 2001). Wound sites were the main sites of penetration of postharvest fungal pathogens such as *B. cinerea* and *Penicillium expansum.*

2.6.2 UV-C irradiation

The disinfection properties of UV-C irradiation at 254 nm are well known extensively used (Islam *et al.,* 1998; Stevens *et al.,* 1998). In addition to its direct lethal effects, UV-C when applied in low doses has been reported to reduce diseases by inducing physiological reactions (Stevens *et al.,* 1998). UV-C irradiation induces phenylalanine activity and consequently the production of secondary metabolites like flavonoids and furanocoumarins (Chappell and Hahlbrock, 1984; Hahlbrock and Scheel, 1989). Some flavonoids protect plants from excess UV irradiation. Furanocoumarins are generally active in specific plant species (e.g. celery, parsnip and parsley) and could exert fungitoxic activity (Chappell and Hahlbrock, 1984). The phytoalexin scoparone was

induced after UV-C treatment of harvested citrus fruits and its levels were correlated with decay resistance (Ben-Yehoshua *et al.,* 1992). Treatment of cell suspension cultures of parsley with UV light resulted in PAL, 4CL and CHS activation (Chappell and Hahlbrock, 1984). Postharvest treatments with UV-C light have been introduced to reduce disease as being more environmentally friendly than conventional fungicide applications (Liu *et al.,* 1993). However, there is a lack of published research on UV-C application to cut flowers with a view to suppress postharvest diseases.

Application of UV-C at 254 nm at doses ranging from 1.3-40 kJ m^{-2} to harvested tomatoes markedly reduced black mold (*A. alternata*), grey mold (*B. cinerea*) and Rhizopus soft rot (*R. stolonifer*) via defence response induction (Liu *et al.,* 1993). Grey mold caused by *B. cinerea* applied to grapes at different times after harvest was significantly ($P < 0.05$) reduced after UV-C (254 nm) treatment with doses ranging from 0.125-0.5 kJ m^{-2} (Nigro *et al.*, 1998). Reduction of green mould decay caused by *P. digitatum* on grapefruit (Droby *et al.,* 1993) and citrus (Ben-Yehoshua *et al.,* 1992) was attributed to induced resistance in irradiated harvested fruit. PAL and peroxidase activity increased after UV-C (254 nm) treatment at 4.8 kJ m^{-2} (Droby *et al.*, 1993). Stevens *et al.,* (1999) reported that sweetpotato infection by *Fusarium solani* was reduced after UV-C irradiation at 3.6 kJ m^{-2}. Sweetpotato exposure to UV-C (254 nm) at 3.6 kJ m^{-2}, resulted in significant ($P < 0.05$) increase of PAL activity (Stevens *et al.*, 1999). Postharvest decay of 'Empire' apples was reduced by 52% compared to untreated in irradiated with 1.38 kJ m^{-2} UV-C fruits (Wilson *et al.*, 1997). Black rot caused by *Xanthomonas campestris* pv. *campestris* was reduced up to 90% in cabbage after UV-C treatments at 3.6 kJ m^{-2} (Brown *et al.*, 2001). Positive effects of postharvest UV-C (254 nm) treatment on variety of postharvest pathogens (viz. *Monilinia fructicola, Alternaria* spp., *Colletotrichum gloeosporioides, Monilinia* spp., *P. digitatum, A. citri* and *Geotrichum candidum*) infecting fruits (viz. peach, apple, mango, grapefruit and tangerine) have also been reported (Stevens *et al.,* 1996; Gonzalez-Aguilar *et al.*, 2001).

B. cinerea spore inactivation increased with increasing UV-C (254 nm) doses and no *B. cinerea* spores survived after 1 kJ m^{-2} UV-C treatment (Marquenie *et al.,* 2001). The effect of UV-C (254 nm) light on percent germination of *Monilinia fructicola* decreased linearly with increasing UV-C doses ranging from 0 to 20 kJ m^{-2} (Stevens *et al.,* 1998).

2.6.3 Chemical activators

In attempts to find alternative methods for disease control, compounds that activate defence responses have been examined. For example, 2,6-dichloroisoniciotinic acid (INA), salicylic acid (SA), 3-aminobutyric acid (BABA) and benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester (BTH; acibenzolar-S-methyl, CGA 245704 or Bion) have been shown to induce SAR responses in plants, thereby providing prospects for integrated disease management (Kessmann *et al.*, 1996; Ruess *et al.*, 1996). Long-lasting, broad-spectrum protection by inducing defence responses against diseases could have a major impact on socio-environmentally sound horticultural production.

A chemical is generally characterized as a plant activator when it induces SAR and SAR genes and provides protection on the same spectrum of diseases exerted by a wild type host (Kessmann *et al.*, 1996; Ruess *et al.*, 1996). Activators do not normally exert a direct antimicrobial activity against pathogens when used for disease control. Through their mutagenic elicitation effect they could help eliminate the risk for the development of resistant strains of the pathogen.

2.6.3.1 Salicylic acid (SA)

SA plays an important role in SAR-signal transduction after pathogen infection, and has long been known to induce the accumulation of PR-proteins and resistance. Neither SA nor its metabolites seem to have significant direct antimicrobial activity (White 1979). However, further work showed that application of exogenous SA gave only restricted resistance at the site of infection without inducing efficient systemic resistance (White, 1979). In a recent study by Terry (2002), SA showed antifungal *in-vitro* activity against *B. cinerea*. *B. cinerea* colony inhibition increased proportionally with SA concentration. Preharvest treatment of kiwifruit cv. Hayward vines with 0.28 g L^{-1} resulted in a 10-fold rose of PAL activity 2 days after treatment (Reglinski *et al.*, 1997). Pre- and/or postharvest SA application at 2.0 g L^{-1} on mango cv. Kensington Pride fruits tened to reduce infection by *C. gleosporioides* (Zainuri *et al.*, 2001).

However, it was not clear whether SAR was induced. Raskin (1992) found that exogenous SA rapidly became conjugated, preventing its mobility as free salicylate in the phloem. Moreover, phytotoxicity problems were observed after exogenous application of SA to some plants. The latter findings provided evidence that exogenous SA application in practice needs reconsideration (Kessmann *et al.*, 1994).

2.6.3.2. 2,6-dichloroisonicitinic acid (INA)

INA (CGA 41396) was effective against diseases by inducing SAR of plants (Kessmann *et al.*, 1994). INA provided good protection against fungal and bacterial pathogens on cucumber, rice, pepper, pear and tobacco in the greenhouse as well as under field conditions (Kessmann *et al.*, 1994). This compound had no significant *in vitro* activity and was not converted into antimicrobial metabolites *in vivo* (Metraux *et al.*, 1991). In tobacco, pre-inoculation with TMV and treatment with INA induced the same gene families (Ward *et al.*, 1991). A similar correlation between SAR induction by pathogen infection and treatment with INA was observed in cucumber (Metraux *et al.*, 1991) and *Arabidopsis* (Uknes *et al.*, 1992). INA induced β-1,3-glucanases, chitinase and 6-phosphogluconate-dehydrogenase (6-PGD) but it did not induce PAL (Staub *et al.*, 1992). Field and glasshouse experiments with INA resulted in disease severity and lesion size reduction caused by the fungus *Sclerotinia sclerotiorum* on soybean (Dann *et al.*, 1998).

2.6.3.3. Acibenzolar-S-methyl (benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester; CGA 245704; benzothiadiazole or BTH)

The efficacy of acibenzolar has been tested in field, glasshouse and pot trials (Table 2.5). However, there is no published research on the effects of acibenzolar against infection of cut flowers by *B. cinerea*.

Table 2.5: Effects of acibenzolar-S-methyl on different host-pathogen interactions (ns: not shown, na: not applicable).

Host	Pathogen	Induced response	Acibenzolar-S-methyl concentration	Reference
Apple seedlings cv. Golden Delicious	*Erwinia amylovora*	β-1,3-glucanases, peroxidases	0.1-0.2 g AI L^{-1}	Brisset *et al.*, 2000
Cauliflower (*Brassica oleracea* var *botrytis*)	*Peronospora parasitica*	ns	0.0015-0.075 g AI L^{-1}	Godard *et al.*, 1999
Cereals, tobacco	*Erysiphe graminis*, *Septoria* spp., *Puccinia* spp., and *Peronospora hyoscyami* f. sp. *tabacina*	ns	12-30 g AI ha^{-1}	Ruess *et al.*, 1996
Cucumber (*Cucumis sativus* L.)	*Cladosporium cucumerinum*	Acidic peroxidase, class III chitinase and β-1,3-glucanase	32.4 g AI L^{-1}	Narusaka *et al.*, 1999

Host	Pathogen	Induced response	Acibenzolar-S-methyl concentration	Reference
Cucumber (*Cucumis sativus* L.) and Japanese pear (*Pyrus pyrifolia* Nakai var. culta Nakai)	*Cladosporium cucumerinum*, *Colletotrichum lagenarium, Fusarium oxysporum* f.sp. *cucumerinum* , *Corynespora cassiicola, Venturia nashicola, Alternaria alternata, Botrytis cinerea, Didymella bryoniae* and *Gymnosporangium asiaticum*	ns	0.05-0.1 g AI L^{-1}	Ishii *et al.*, 1999
Cauliflower (*Brassica oleracea* var. *botrytis*)	*Peronospora parasitica*	β-1,3-glucanase. PR-1 and PR-5	0.05 g AI L^{-1}	Ziadi *et al.*, 2001
Melons cv. Early Yellow Hami	*Fusarium* spp., *Alternaria* spp., *Rhizopus* spp. and *Trichothecium* sp	ns	0.025 or 0.05 g AI L^{-1}	Huang *et al.*, 2000
Parsley cells (*Petroselinum crispum* L.)	With or without elicitor (Pmg)	PAL, coumarins	0.32-6.48 g AI L^{-1}	Katz *et al.*, 1998
Pepper (*Capsicum annuum* L.)	*Xanthomonas campestris* pv. *vesicatoria*	ns	1.25-5 g AI L^{-1}	Buonaurio *et al.*, 2002
Rose plants var. 'Iris Gee'	*Diplocarpon rosae*	β-1,3-glucanase. chitinase	3.24 g AI L^{-1}	Suo and Leung, 2001

Host	Pathogen	Induced response	Acibenzolar-S-methyl concentration	Reference
Soybean seedlings	*Sclerotinia sclerotiorum*	ns	0.035-0.375 g AI L^{-1}	Dann *et al.*, 1998
Strawberry plants cvs. Elsanta and Andana	*Botrytis cinerea*	ns	0.25-2 g AI L^{-1}	Terry and Joyce, 2000
Sunflower plants (*Helianthus annuus* L. cv. Ala)	*Plasmopara helianthi*	ns	0.1-0.2 g AI L^{-1}	Tosi *et al.*, 1999
Tobacco plants cv. Kutsaga Mammoth 10	*Pseudomonas syringae* pv *tabaci, Thanatephorus cucumeris*, and *Cercospora nicotianae*	ns	0.05-30 g AI L^{-1}	Cole, 1999
Tomato plants (*Lycopersicon esculentum*)	*Fusarium oxysporum* f.sp. *radicis-lycopersici*	Callose enriched wall appositions phenolic compounds	97.2 g AI L^{-1}	Benhamou and Belanger, 1998
Tomato plants cv. Vollendung (*Lycopersicon esculentum*)	Cucumber mosaic virus (CMV)	ns	0.1 mM	Anfoka, 2000
Wheat	*Erysiphe graminis* f.sp. *tritici*	WCI genes (1-5)	0.3 mM	Gorlach *et al.*, 1996

Acibenzolar-S-methyl, was introduced as a potent inducer of SAR and treated plants were resistant to the same spectrum of diseases as plants activated naturally (Kessmann *et al.*, 1996; Friedrich *et al.*, 1996). Although acibenzolar and its metabolites exhibited no direct antimicrobial activity towards plant pathogens tested, they induced the same biochemical processes in the plant as those observed after natural activation of SAR (Friedrich *et al.*, 1996; Lawton *et al.*, 1996). The compound, which was inactive in plants that do not express the SAR-signaling pathway, required a lag time of approximately 30 days between application and protection (Lawton *et al.*, 1996).

2.6.3.4. Jasmonates

Efficacy of jasmonates has been tested in field, glasshouse and pot trials (Table 2.6). Little work has been done on the effect of jasmonates against *B. cinerea* infection of cut flowers. JA and its esterified derivative methyl jasmonate (MeJA) gave systemic protection to various rose cultivars (e.g. Mercedes, Europa, Lambada, Frisco, Sacha and Eskimo) against *B. cinerea* (Meir *et al.*, 1998). MeJA applied as a postharvest pulse, significantly reduced *B. cinerea* lesion size on detached rose petals. In the same study, MeJA at concentrations of 100-400 μM showed *in-vitro* antifungal activity on *B. cinerea* spore germination and germ-tube elongation. JA and MeJA were also tested on grapefruit for suppressing postharvest green mold decay [*Penicillium digitatum* (Pers.:Fr.) Sacc.] (Droby *et al.*, 1999). Studies showed that 50 μM and 1 μM MeJA concentrations were effective against the disease and that the reduction in the decay was the same at incubation temperatures of 2 or 20°C. Moreover, as the *in-vitro* tests showed no direct antifungal activity of JA and MeJA, it was suggested that the disease suppression was achieved via natural resistance induction (Droby *et al.*, 1999). According to Cohen *et al.* (1993), JA and MeJA applied at 300-4800 μM to potato cv. Bintje or Alpha and tomato cv. Baby plants as foliar spray protected them against *P. infestans* infection. In the same study it was shown that jasmonates did not exert any antifungal activity *in-vitro*.

Table 2.6: Effects of methyl jasmonate on different host-pathogen interactions in summary. (ns: not shown, na: not applicable).

Host	Pathogen	Induced response	Methyl jasmonate concentration	Reference
Arabidopsis (*Arabidopsis thaliana*)	*Botrytis cinerea, Alternaria brassicicola, Plectosphaerella cucumerina*	ns	0.5-50 μM and 0.001-1 μL/L air	Thomma *et al.*, 2000
Arabidopsis (*Arabidopsis thaliana*)	*Alternaria brassicicola*	PDF1.2	45 μM	Penninckx *et al.*, 1996
Grapefruit (*Citrus paradisi*) var. 'Marsh Seedless	*Penicillium digitatum*	ns	1-50 μM	Droby *et al.*, 1999
Large number of species	na	PPO	na	Constabel and Ryan, 1998
Potato plants (*Solanum tuberosum*)	*Phytophthora infestans*	phytoalexins	1-10 μM	Il'inskaya *et al.*, 1996
Rose plant (*Rosa hybrida*)	*Botrytis cinerea*	ns	50-600 μM	Meir *et al.*, 1998
Tobacco cell cultures	na	β-glucuronidase (GUS), osmotin protein	0.045-4550 μM	Xu *et al.*, 1994

Host	Pathogen	Induced response	Methyl jasmonate concentration	Reference
Tobacco cv. Xanthi-nc NN	*Phytophthora parasitica* var. *nicotianae*, *Cercospora nicotianae* and TMV	β-glucuronidase (GUS)	45 μM	Mitter *et al.*, 1998
Tomato plants (*Lycopersicon esculentum*)	*Helicoverpa zea*, *Spodoptera exigua*	PPO, POD, LOX and PIs	0.1-10 mM	Thaler *et al.*, 1996
Tomato plants (*Lycopersicon esculentum*)	*Spodoptera exigua*, *Pseudomonas syringae* pv. *tomato*	PPO	1 mM	Thaler *et al.*, 1999

Peaks in LOX activity at 2 and between 4 to 8h following MeJA treatment of tobacco cells have also been reported (Dubery *et al.*, 2000). Treatment of *Arabidopsis* plants with MeJA resulted in reduced disease development of *A. brassicicola, B. cinerea* and *Plectosphaerella cucumerina* (Thomma *et al.*, 2000). Application of gaseous MeJA to plants resulted in a greater disease reduction compared to that on plants sprayed with MeJA or treated with INA. Gaseous MeJA protected SA-degrading transformant *NahG* plants, suggesting that gaseous MeJA induced a non-SA dependent systemic response (Thomma *et al.*, 2000). Combination of acibenzolar and JA was tested against bacterial and insect attack on field grown tomato plants (Thaler *et al.*, 1999). Two signaling pathways, one involving SA and another involving JA were proposed to provide resistance against pathogens and insect herbivores, respectively (Thaler *et al.*, 1999).

Systemic activation of a plant defensin gene (*PDF1.2*) was activated after treatment with MeJA of *Arabidopsis* leaves (Penninckx *et al.*, 1996) and tobacco plants (Mitter *et al.*, 1998). A 5-kD plant defensin was purified from *Arabidopsis* leaves challenged with the fungus *A. brassicicola* and was shown to possess antifungal activity *in-vitro* (Penninckx *et al.*, 1996). The corresponding plant defensin gene (*PDF1.2*) was induced after treatment of *Arabidopsis* leaves with MeJA and ethylene but not with SA or INA. Expression of *PDF1.2* gene was evident in *nahG* plants that do not have the ability to synthesize SA and PR-1 protein when infected (Lawton *et al.*, 1995). This effect indicated two separate classes of antifungal proteins (PR-1 and plant defensins) induced by different signaling pathways: viz. SA-dependent and JA/Ethylene-dependent pathways, respectively (Penninckx *et al.*, 1996).

2.7 CONCLUDING REMARKS

Cut freesia, gerbera, rose and Geraldton waxflower production suffers from severe *Botrytis* infection problems (Elad, 1988; Salinas *et al.*, 1992; Salinas and Verhoeff, 1995; Tomas *et al.*, 1995) that lead to substantial economic losses (D. Zwetsloot, pers. comm., 2000). Pre- and/or postharvest application of defence inducers could offer an alternative avenue to socially and environmentally less desirable control by

conventional fungicides. Strategies to control postharvest flower diseases could be based on a model proposed by Joyce and Johnson (1999) (Figure 2.6).

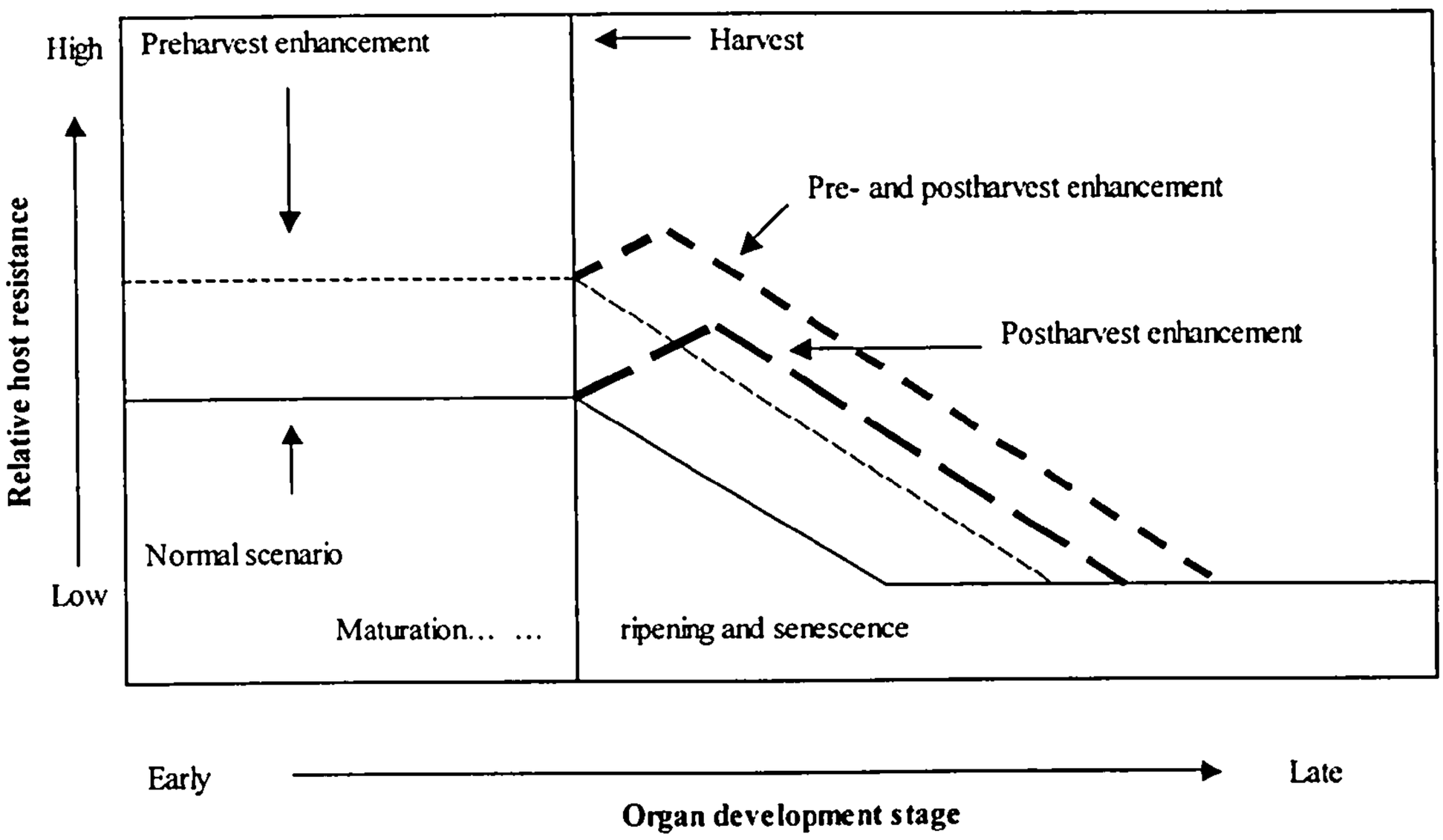

Figure 2.6: Schematic model showing the possible enhancement of natural defence responses of harvested horticultural crops (after Joyce and Johnson, 1999).

According to Joyce and Johnson (1999), natural defence response enhancement with defence inducers may be achieved by a pre- or a postharvest treatments or by combined pre- and postharvest treatments. This enhancement could lead to an increased resistance against postharvest diseases of harvested horticultural products during their ripening and senescence (Joyce and Johnson, 1999). Defence response enhancement might be achieved, for instance, by MeJA, acibenzolar, UV-C and/or *A. pullulans* treatment. This elicitation would lead to altered regulation via signaling and the subsequent production of inhibitory compounds and secondary metabolites against the pathogen(s). These prospects are examined for freesia in the following experimental chapters.

CHAPTER 3

ENVIRONMENTAL FACTORS THAT POTENTIALLY AFFECT INFECTION OF CUT FREESIA FLOWERS BY *B. CINEREA*

3.1 REJECTIONS IN RELATION TO PRE-HARVEST ENVIRONMENTAL CONDITIONS

3.1.1 Introduction

There have been few attempts in the past to forecast *Botrytis* disease incidence in the field or in storage on the basis of environmental data. Jarvis (1964) suggested that forecasting would be challenging due to the complexity of meteorological, edaphic and biotic factors that affect plant-pathogen interactions. Nonetheless, *Botrytis* diseases are generally favoured by cool and wet or humid conditions (Jarvis, 1977). Forecasts of epidemics generally utilise preceding weather data over a period not less than 48h as this is the period of time needed for infection to occur (Jarvis, 1977; 1980b). Monitoring the dispersal of conidia and airborne inoculum population cannot forecast an epidemic if it is not correlated with weather records (Jarvis, 1980b). Airborne *B. cinerea* inoculum levels and lesion development on flowers inside gerbera (Kerssies, 1993) and rose (Kerssies *et al.*, 1995) glasshouses in The Netherlands did not follow seasonal patterns. However, airborne inoculum levels inside rose glasshouses were positively correlated with the number of lesions caused by *B. cinerea* on flowers (Kerssies *et al.*, 1995). Positive correlations have also been reported between lesion numbers on gerbera (Kerssies, 1993) and rose (Kerssies *et al.*, 1995) flowers and relative humidity (RH). In contrast, Kerssies and Frinking (1996) found no correlation between glasshouse environmental conditions and incidence of *B. cinerea* on gerbera and rose flowers of gerbera and rose flowers.

By air or by truck are the two common modes of transport for ornamentals. Trucks are often equipped with temperature and sometimes humidity control. There are relative advantages and disadvantages with both means of transport. Shipping flowers by

air has the advantage of reducing transit time for long distances, including overseas travel. However, it seldom offers temperature control (Maxie *et al.*, 1973). In contrast, fixed refrigeration units on trucks can provide ideal temperature conditions for the maintenance of flower quality. According to Halevy *et al.* (1978) and Farnham *et al.*, (1979), the longevity and quality of flowers sent by refrigerated trucks in a transit study from California to Florida were comparable to or even better than for air-shipped flowers. However, during export handling of harvested flowers in un-refrigerated environments, the optimum temperature and relative humidity conditions are seldom achieved. Condensation within cartons can occur as a result of product transpiration and temperature fluctuations (Joyce and Patterson, 1993). Van der Sman *et al.* (1996) reported infection of cut roses by *B. cinerea* as a result of temperature fluctuations during transport. Moreover, increased *B. cinerea* disease incidence was observed on cut roses after re-warming of flowers inside the packing box (van der Sman *et al.*, 1996).

This study was undertaken in an attempt to explain the variation accross the year 2000 in *B. cinerea* incidence on cut freesia flowers as noted by the UK importer Zwetsloots & Sons Ltd. (Sandy, Bedfordshire, UK) throughout the year. Climate records from the freesia production glasshouse in De Lier (The Netherlands) and environmental records from the nearest weather station at Vlissingen were collated. These data were correlated to cut freesia rejections in the UK. In addition, freesia handling chain temperature and relative humidity data were collected to complement preharvest environmental data.

3.1.2 Materials and Methods

3.1.2.1 Plant material

Freesia hybrida vars. 'Avila', 'Vivaldi', 'Argenta', 'Volante', 'Aristo', 'Blue moon', 'Elegance', 'Oberon' and 'Elysee' were grown by Arie Stolk in glasshouses for commercial cut flower production in De Lier (The Netherlands).

3.1.2.2 Glasshouse environmental conditions

Air temperature (°C) and relative humidity (RH; %) data for the year 2000 were collected for correlation with rejection data provided by Zwetsloots & Sons Ltd (Sandy, UK). Temperature and RH inside the glasshouse were measured by wet-dry bulb thermometer-sensors (Priva CD 750 jr. 1992, The Netherlands) placed approximately 50 cm above the crops (Plate 3.1).

Plate 3.1: Wet-dry bulb thermometer-sensors (Priva CD 750 jr. 1992, The Netherlands) hung approximately 50 cm above freesia crops inside the glasshouse in De Lier, The Netherlands.

Data were recorded daily at 6h intervals. A total of seven temperature-humidity sensors were evenly distributed inside the glasshouse area. Each sensor was for *ca.* 2,500 m^2 of the total glasshouse area of 17,500 m^2. Data were logged by a computer and printed out. Daily means of four readings (day and night) of temperature and RH for each of the seven loggers inside the overall glasshouse area were used as the database for correlation analyses and graphic presentation. Vapour pressure deficit (VPD; kPa) values were estimated from temperature and RH means using a psychrometric chart (IHVE, 1013.25 mbar).

3.1.2.3 Weather data

Regional environmental data were also obtained from the meteorological station at Vlissingen, The Netherlands (KMNI: Koninklijk Netherlands Meteorolisch Instituut) and correlated with disease incidence data recorded at Zwetsloots & Sons. Three-day-mean values were calculated, for temperature and sun duration, data preceding harvest as recorded at the Vlissingen weather station. Rainfall and wind speed were also calculated as both could potentially affect inoculum dispersal and RH levels inside the glasshouse, respectively.

3.1.2.4 Freesia rejection data

Each freesia flower consignment imported into the UK was examined by quality control personnel at Zwetsloots & Sons. Rejection of either part or whole consignments was based on specking incidence on the flowers. One to three disease spots on the first flower (i.e. most mature) in 50% of the total amount of flower stems per bucket (i.e. 200 flowers) resulted in rejection of the whole bucket. Rejection dates and rejected stem numbers were collated and correlated with preharvest environmental data.

3.1.2.5 Experimental

Data means of temperature and relative humidity were calculated on the basis of records obtained from seven humidity-temperature sensors placed in the glasshouse. Three-day-mean values were calculated from the daily mean values (i.e. four readings) over a 3-day-period preceding harvest. With 48h being the minimum time for infection to occur in glasshouse crops (Jarvis 1980), a 72h period would accommodate environmental conditions during the infection risk period.

Harvested freesia flowers were held inside the glasshouse area and then inside the auction area for approximately 12h until they were loaded into trucks for transport to the UK (D. Zwetsloot, pers. comm., 2000). The proportion (%) of rejected individual stems out of the total stem number ordered by Zwetsloots & Sons per month was calculated.

All individual temperature and RH 3-day-means before rejections for each month were averaged to give the monthly temperature, RH, VPD, sun duration, rainfall and wind speed 3-day-mean values. This was to additionally check for possible correlations. Linear ($y = y_o + ax$) and quadratic ($y = y_o + ax + bx^2$) regression analyses between 3-day monthly-means of temperature, RH, VPD, sun duration, rainfall and wind speed and percentage of rejected stems per month were performed over the year in 2000.

3.1.2.6 Handling chain monitoring

Temperature and RH measurements during the postharvest handling of cut freesia flowers were recorded twice over 2 years (25th November 1999 and 2nd February 2001). Monitorings started from the grower in The Netherlands and ended to Zwetsloots & Sons in the UK. Environmental conditions were monitored with Gemini 'Tinytalk' (Gemini Dataloggers Ltd., Chichester, UK) (Plate 3.2) loggers which were programmed to record temperature and RH at 5, 10, 30 or 60 min intervals.

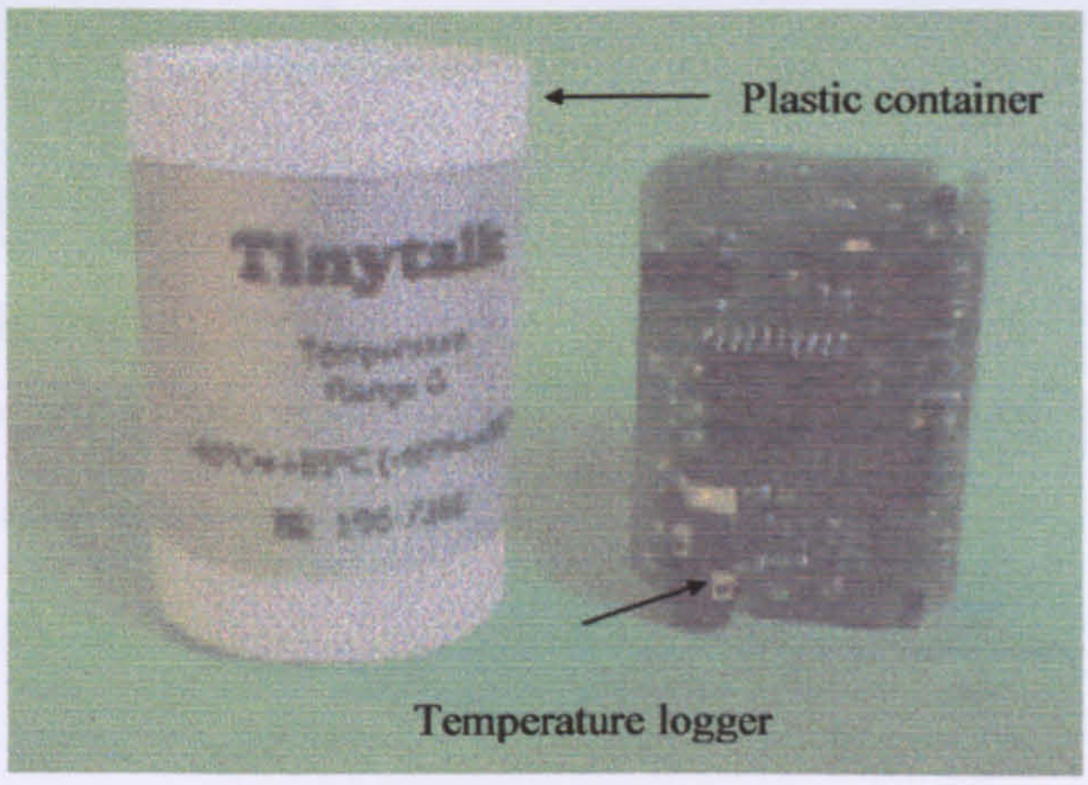

Plate 3.2: A Gemini 'Tinytalk' temperature logger as used for handling chain monitoring.

Loggers were attached to the second or third shelf of the transport trolleys, carrying buckets of freesia flowers (Plate 3.3). Recorded data were downloaded using Gemini 'Tinytalk' software and then transferred into Sigmaplot 2000 (Chicago, IL, USA) for graphic presentation.

Plate 3.3. Trolley with freesia flowers at the Westland auction in The Netherlands. Freesia bunches are divided in 100 stems and are put into buckets with holding solution. Arrows indicate logger attachment points.

3.1.3 Results

Four peaks of rejected stems per monthly order were recorded in year 2000, two in spring (i.e. April and May), one in summer (i.e. June) and one in autumn (i.e. October) (Figure 3.1A). The highest proportions of rejected stems, based on the total number of

stems ordered per month, were recorded in April (2.9%), May (3.3%), June (2.0%) and October (3.2%). Similarly, four peaks in rejected stem numbers per month were recorded in 2000, one in spring, two in summer and one in Autumn (Figure 3.1B).

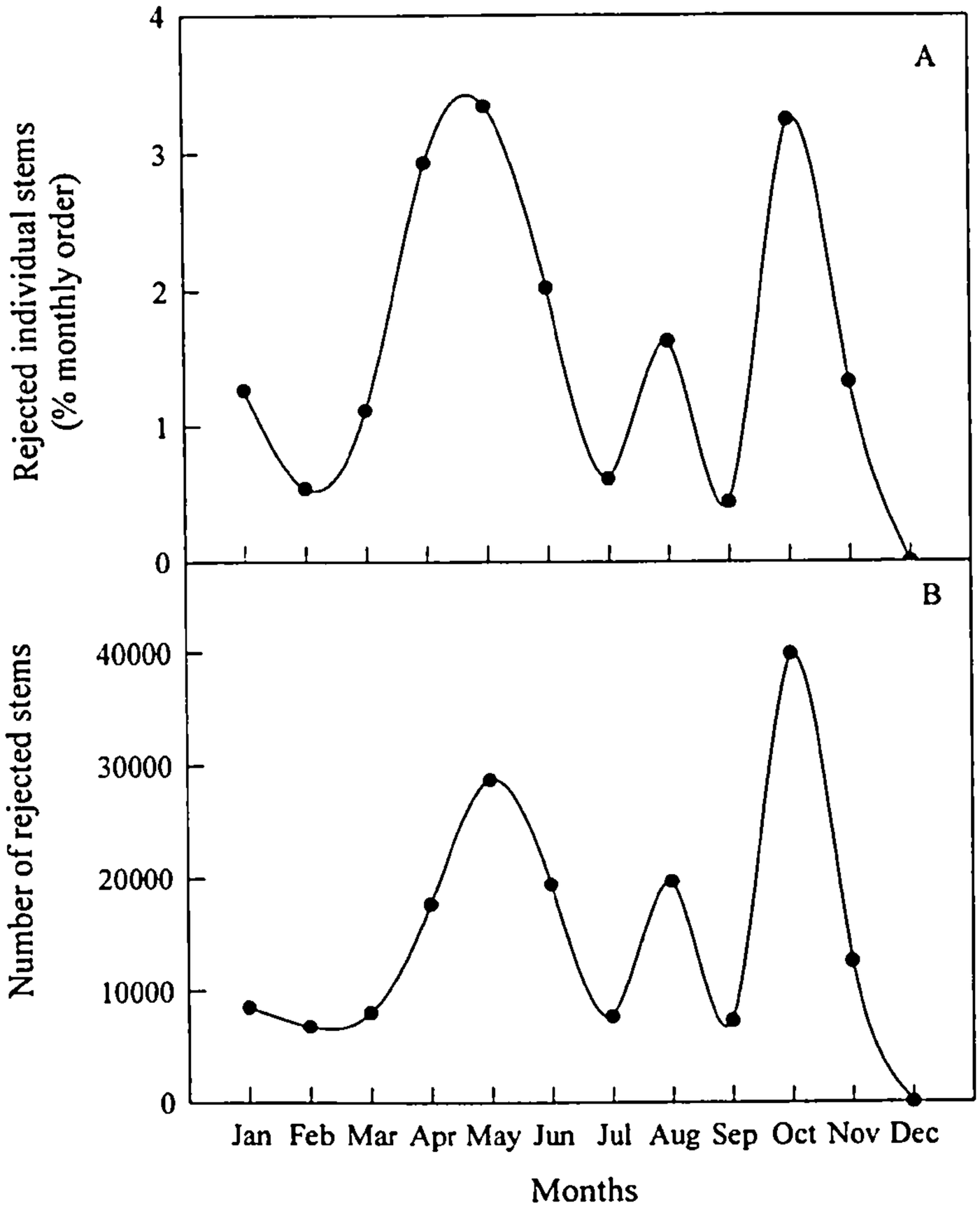

Figure 3.1: Proportion (%) (A) and number (B) of freesia flower stems rejected monthly in the UK during the year 2000. The flowers were imported from the Westland auction in The Netherlands.

The highest rejected stem numbers were recorded in May (28,664), June (19,356), August (19,608) and October (39,774). These high numbers resulted in substantial economic losses for both the grower and the importer (D. Zwetsloot, pers. comm., 2000). Based on the weather data in the year 2000, monthly 3-day temperature means ranged between 6.3 and 19.0°C (Figure 3.2 A). Relatively higher proportional rejections occurred at temperatures ranging from 10 to 16°C (Figure 3.2 A). Sun duration values

ranged between 1.5 and 9.2 h (Figure 3.2 B). Rainfall values ranged from 0 to 4.3 mm (Figure 3.2 C). Wind speed values ranged between 3.7 and 9.8 m sec^{-1} (Figure 3.2 D).

Finally, temperature, RH and VPD means inside the glasshouse ranged between 9.8 and 20.2°C, 76.9 and 95.7%, and 1.2 and 1.9 kPa, respectively (Figure 3.3 A, B, C). Comparatively higher proportions of rejected stems were associated with glasshouse temperatures ranging from 13 to 17°C (Figure 3.3 A).

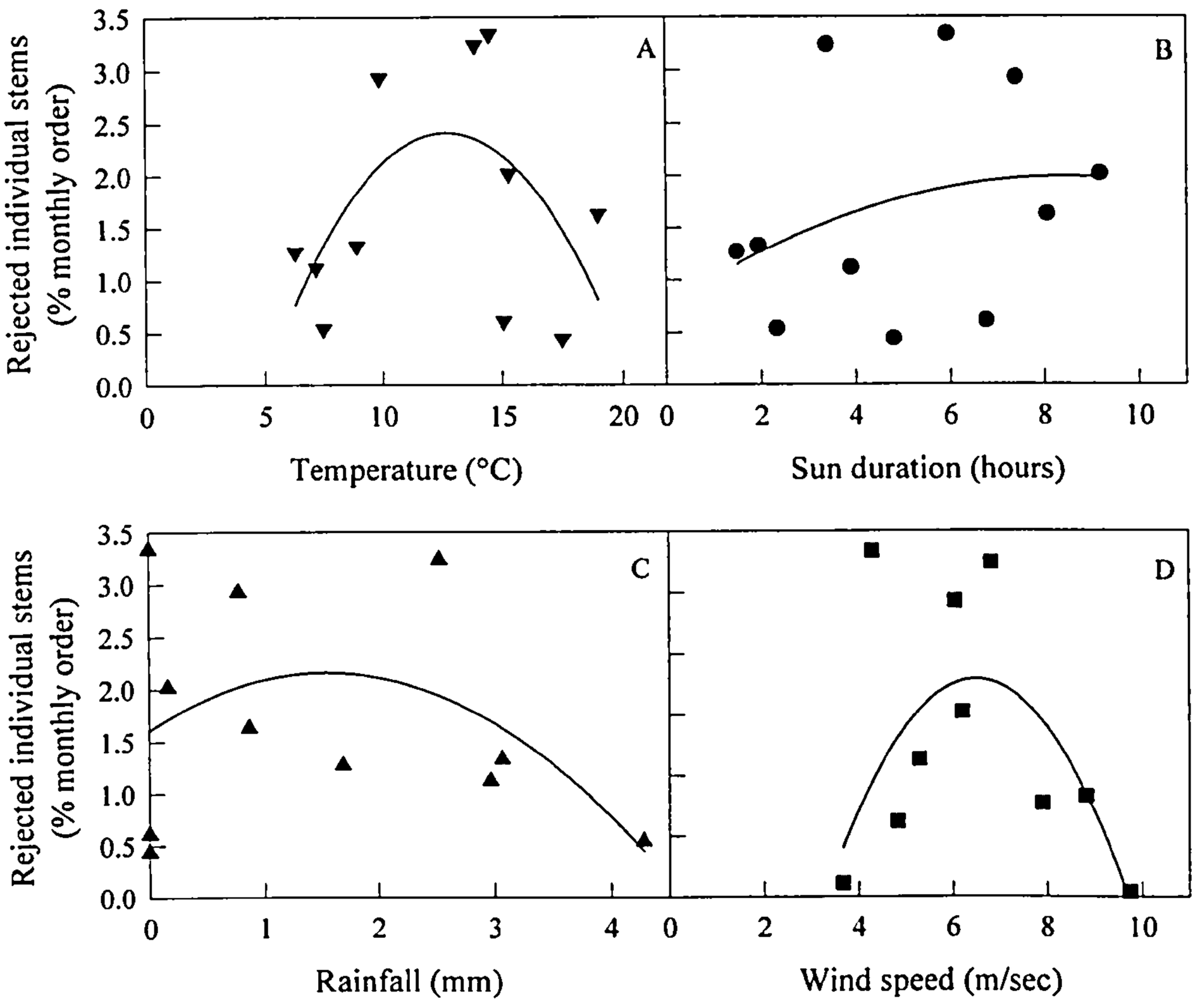

Figure 3.2: Proportion (%) of freesia stems from The Netherlands rejected monthly in the year 2000 in the UK as a function of temperature (A), sun duration (B), rainfall (C) and wind speed (D) in the region of Vlissingen, Holland. The horizontal axis data are 3-day-means preceding harvest. Correlations: A: $y = -4.08 + 1.02x - 0.04x^2$, $R^2 = 0.3$, $P = 0.24$. B: $y = 0.75 + 0.29x - 0.017x^2$, $R^2 = 0.09$, $P = 0.7$. C: $y = 2.24 + 1.02x - 0.17x^2$, $R^2 = 0.31$, $P = 0.23$. D: $y = 1.6 + 0.71x - 0.23x^2$, $R^2 = 0.18$, $P = 0.45$

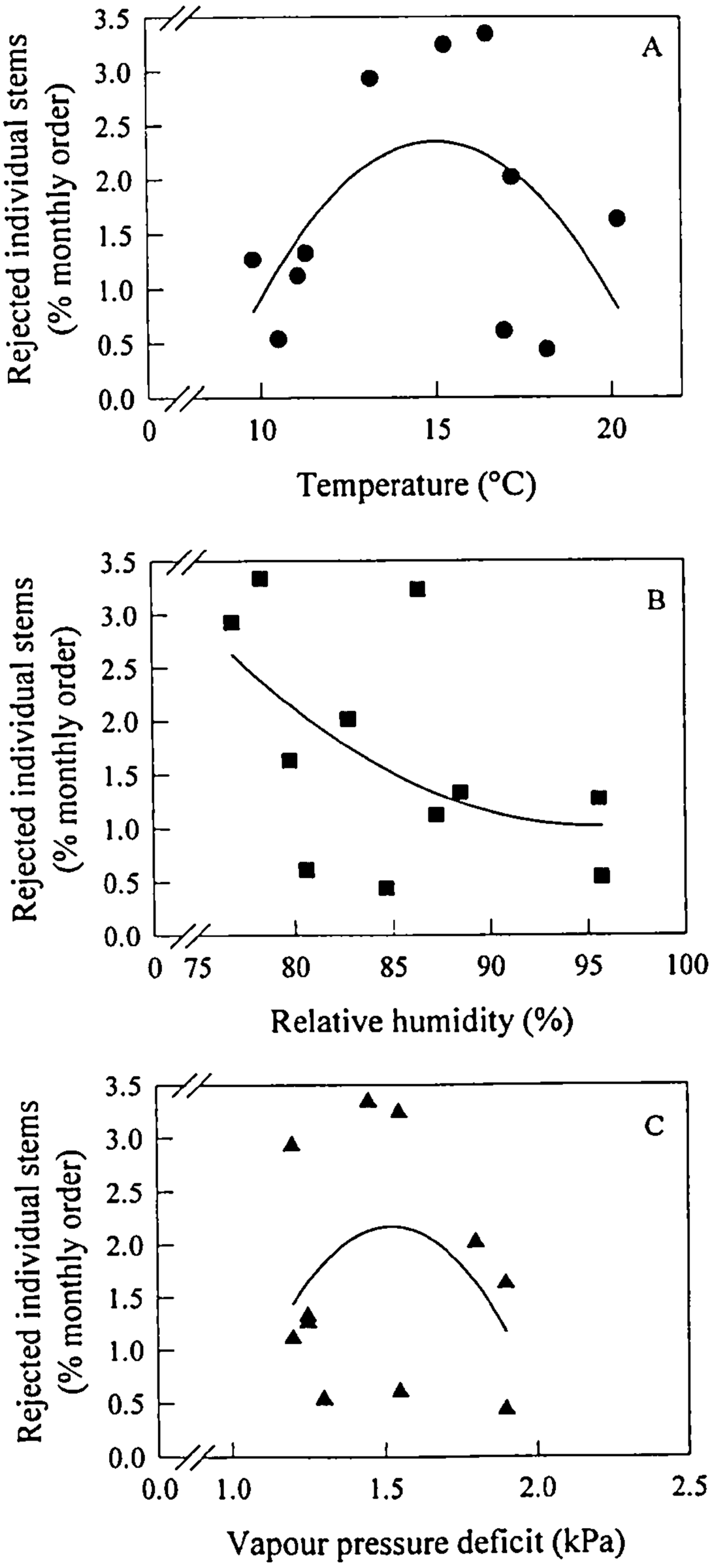

Figure 3.3: Proportion (%) of freesia stems from The Netherlands rejected monthly in the year 2000 in the UK as a function of temperature (°C) (A), relative humidity (RH) (%) (B) and vapour pressure deficit (VPD) (kPa) (C) inside the glasshouse in Holland. The horizontal axis data are 3-day-means preceding harvest. Correlations: A: $y = -10.59 + 1.72x - 0.06x^2$, $R^2 = 0.28$, $P = 0.27$. B: $y = 0.27 - 0.9x + 0.005x^2$, $R^2 = 0.27$, $P = 0.29$. C: $y = -14.01 + 21.25x - 6.98x^2$, $R^2 = 0.12$, $P = 0.57$.

There were no significant ($P > 0.05$) linear ($y = y_0 + ax$) or quadratic ($y = y_0 + ax + bx^2$) correlations between temperature, RH or VPD inside the glasshouse versus proportions of monthly rejected freesia stems (Figure 3.3). In addition, no significant ($P > 0.05$) linear or quadratic correlation was found between temperature, sun duration, rainfall and wind speed in the area where the glasshouse was situated in The Netherlands versus the proportion of monthly freesia stems rejected in the UK (Figure 3.2).

Temperature and RH levels throughout the freesia handling chain varied substantially from the glasshouse in The Netherlands to the wholesaler in the UK (Figure 3.4 A, B, C and D). In the first monitoring run on 25th of November 1999, temperatures inside the glasshouse, the auction area, the truck and the importer's facilities fluctuated between 11.7-12.0, 12.8-15.3, 8.2-13.8 and 6.3-8.0°C, respectively (Figure 3.4 A). Similarly, RH in these areas fluctuated between 68-95, 71-90, 78-94 and 75-79%, respectively (Figure 3.4 B). In the second monitoring run on 2nd of February 2001, temperatures inside the glasshouse, the auction area, the truck and the importer's facilities fluctuated between 7.0-10.6, 10.2-12.2, 9.8-19.8 and 10.2-10.6°C, respectively (Figure 3.4 C). RH ranged between 83-100, 86-96, 63-97 and 96-98%, respectively (Figure 3.4 D). Overall, temperature fluctuated from 6.3-15.3°C in the first monitoring and from 7.0-19.8°C in the second monitoring (Figure 3.4 A and C). Overall, RH fluctuated from 68-95% and from 63-100% in the two monitoring periods, respectively (Figure 3.4 B and D). Quality control personnel at Zwetsloots & Sons inspected freesia consignments 55 and 90h after harvest on the 25th of November 1999 and on the 2nd of February 2001, respectively (Figure 3.4).

In ancillary work, temperature and RH levels were monitored during freesia transportation from Westland auction in The Netherlands to Zwetsloots & Sons Ltd in the UK. In a third monitoring run on 5th of September 2001, temperatures inside the truck dropped from 16.0-13.5°C (Figure 3.5 A). RH inside the truck fluctuated from 70.8-75.9% (Figure 3.5 B). In the fourth monitoring run on 7th of November 2001, temperatures inside the truck during freesia transportation dropped from 14.5-8.0°C (Figure 3.5 C). RH inside the truck ranged from 69.8-96.0% (Figure 3.5 D).

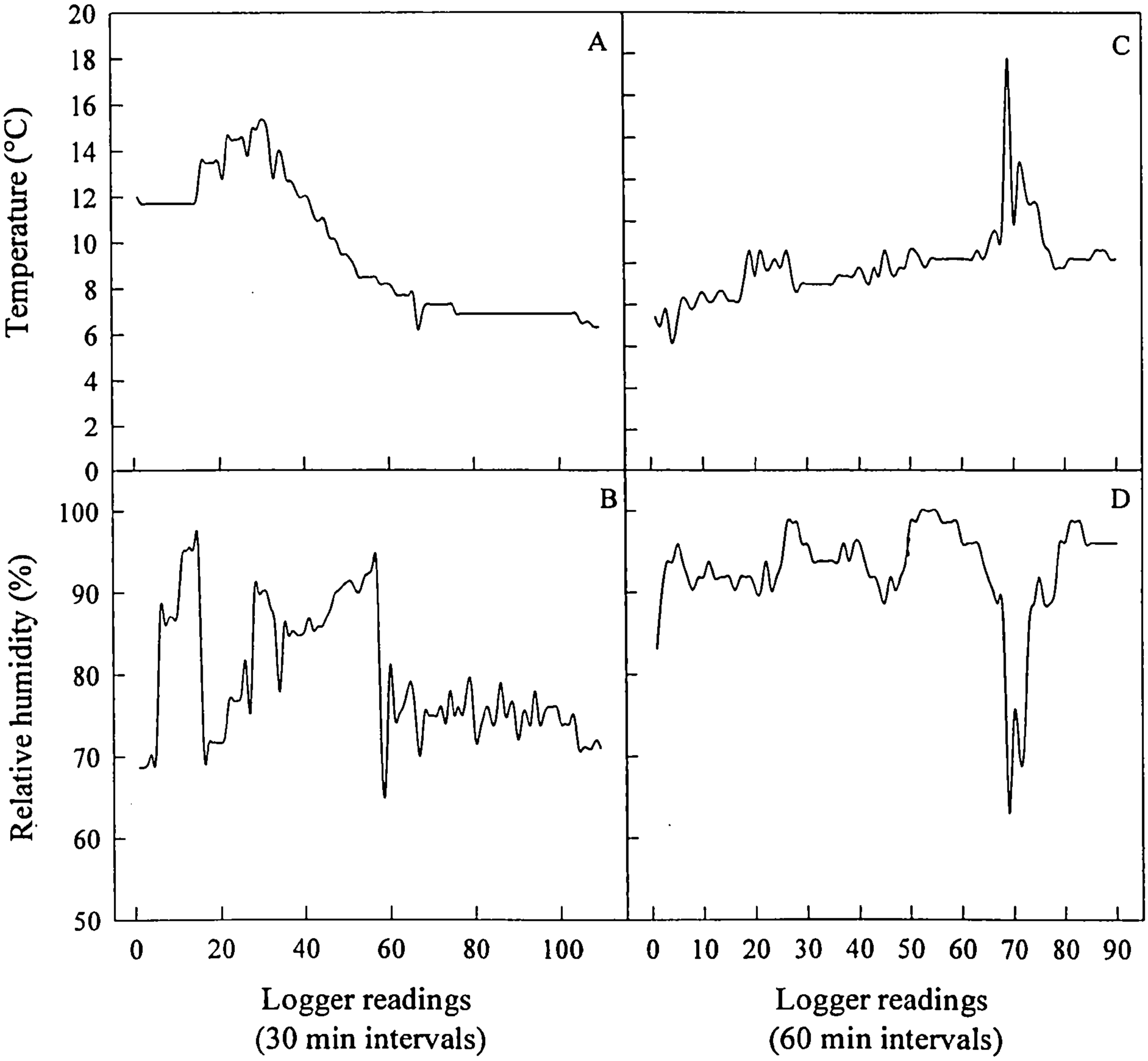

Figure 3.4: Temperature (°C) (A, C) and RH (%) (B, D) levels recorded during cut freesia handling on the 25th November 1999 (A, B) and on 2nd February 2001 (C, D). First monitoring was commenced on the 25th November 1999 from the glasshouse in The Netherlands (10:03), to Westland Flower Auction (18:03), inside the truck driving to UK (03:03), and finally, to Zwetsloots & Sons Ltd. in the UK (18:03). Second monitoring was commenced on the 2nd February 2001 from the glasshouse in The Netherlands (16:07), to Westland Flower Auction (05:07), inside the truck driving to UK (12:07), and finally, to Zwetsloots & Sons Ltd. in the UK (01:07).

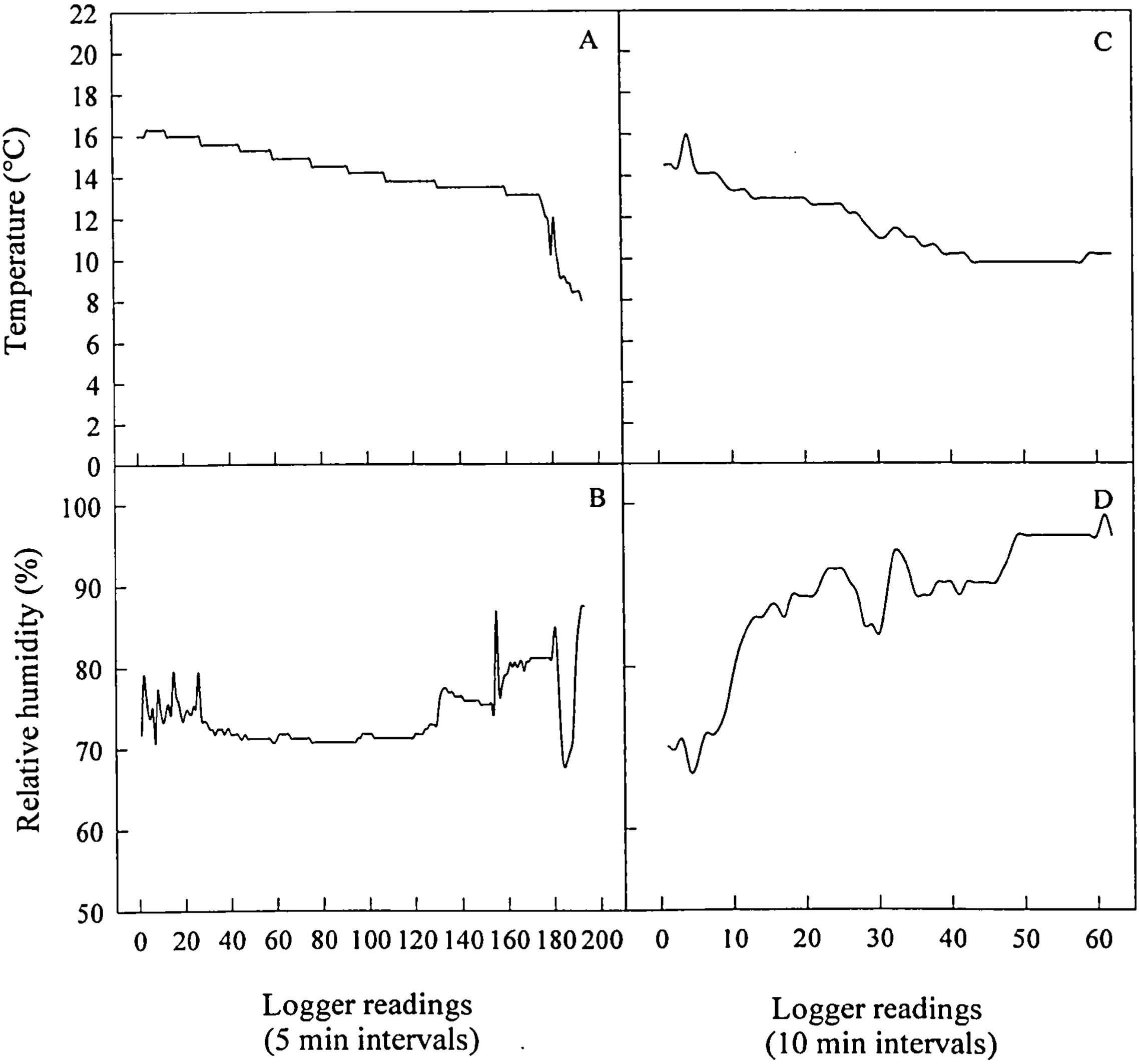

Figure 3.5: Temperature (°C) (A, C) and RH (%) (B, D) levels recorded during transportation on the 5th of September 2001 (A, B) and on the 7th of November 2001 (C, D). First monitoring was commenced on the 5th of September 2001 from the Westland Flower Auction in The Netherlands (16:02) and inside the coldrooms at Zwetsloots & Sons Ltd. after off-loading (04:57). Second monitoring was commenced on the 7th of November 2001 from the Westland Flower Auction in The Netherlands (14:07) and inside the coldrooms at Zwetsloots & Sons Ltd. after off-loading (03:07).

3.1.4 Discussion

The results of the present study showed that in the year 2000 relatively higher monthly proportions of freesia flower stems rejected due to *B. cinerea* infection were recorded during spring (April-May), early summer (June) and autumn (October). Kerssies (1993) reported peaks in *B. cinerea* lesion numbers on gerbera petals during autumn and early summer 1988-89 in a glasshouse in Holland. Similar patterns were observed in rose glasshouses in The Netherlands where the mean number of *B. cinerea* colonies on selective agar media spore traps evenly distributed inside the glasshouse peaked approximately 170, 260, 270 and 300 days after 1st January 1991 (i.e. in June, September and October) and approximately 20, 160, 180, 250, 290 and 320 days after 1st January 1992 (i.e. in January, June, September, October and November; Kerssies *et al.*, 1995). However, Kerssies *et al.* (1995) found no strong correlation between the number of *B. cinerea* colonies on agar selective *B. cinerea* media traps and the number of *B. cinerea* lesions on rose petals. The spring peak in disease incidence found in the present study was not observed in either gerbera or rose glasshouses (Kerssies, 1993; Kerssies *et al.*, 1995).

Based on the results of the present study, comparatively higher proportions of rejected freesia stems were associated with glasshouse temperatures ranging from 13-17°C. According to Bulger *et al.* (1987), *B. cinerea* was able to infect strawberry flowers within a broad range of temperatures from 5-25°C. In the presence of a water film on the surface of gerbera flowers, *B. cinerea* conidia were able to germinate and produce visible lesions at temperatures ranging from 4-25°C (Salinas *et al.*, 1989). However, even in the absence of a water film on the surface of rose petals, 60% of the inoculated flowers produced visible lesions after 48h of incubation at 15°C and 94% RH (Williamson *et al.*, 1995). Temperature has a limited effect on *B. cinerea* development since low temperatures only slow down but do not stop *B. cinerea* growth (Salinas *et al.*, 1989; Salinas and Verhoeff, 1995). A correlation between temperature and disease incidence could also be attributed to physiological responses of the host to temperature (Kerssies, 1994). High temperatures result in turgor increases which may cause nutrient, sugars and salt leakage from host cells. According to Blakeman (1980) and Salinas *et al.* (1989) such leakage could stimulate *B. cinerea* conidial germination.

In the present study, there were no significant ($P > 0.05$) linear ($y = y_0 + ax$) or quadratic ($y = y_0 + ax + bx^2$) correlations between 3-day means of temperature, RH and VPD, sun duration, rainfall and wind speed versus the proportion of freesia stems rejections. The effect of temperature on *B. cinerea* infection incidence on strawberry and gerbera flowers in the field or in glasshouses has been reported by Bulger *et al.*, 1987 and Kerssies, 1994. Temperatures of 20°C promoted the highest *B. cinerea* incidence compared to 10 or 15°C. Positive correlations between *B. cinerea* lesions on gerbera and rose petals and RH have also been found in the past (Kerssies, 1993; Kerssies *et al.*, 1995). However, petal wetness or the presence of a water film on host surfaces rather than RH level might be more important for promotion of *B. cinerea* conidial germination and host infection (Salinas *et al.*, 1989; Salinas and Verhoeff, 1995).

3.2 EFFECTS OF TEMPERATURE, RELATIVE HUMIDITY AND INOCULUM LEVEL ON POST-HARVEST INFECTION OF FREESIA FLOWERS BY *BOTRYTIS CINEREA*

3.2.1 Introduction

Temperature and RH affect *B. cinerea* incidence on cut flowers after harvest (Salinas *et al.*, 1989; Salinas and Verhoeff, 1995). Maintaining Geraldton waxflower at temperatures below 10°C and RH below 90% reduced postharvest *B. cinerea* severity and associated flower abscission (Taylor *et al.*, 1997). Humidity is generally regarded as an extremely important factor for *B. cinerea* establishment (Jarvis, 1977). Artificially inoculated gerbera flowers incubated at room temperature and 100% RH showed typical necrotic lesions 7h after the inoculation event. However, no visible symptoms appeared when flowers were incubated at room temperature and 50-70% RH (Salinas *et al.*, 1989). *B. cinerea* dry conidia were able to germinate and infect rose petals when the RH of the ambient air was as low as 94% (Williamson *et al.*, 1995).

There is apparently no published research on factors affecting postharvest freesia infection by *B. cinerea*. In the present study the effects of temperature, RH and inoculum

level on post-harvest infection of freesia cut flowers by *B. cinerea* were investigated. Histological studies of the freesia-*B. cinerea* interaction were also conducted.

3.2.2 Materials and methods

3.2.2.1 Plant material

Freesia var. 'Cote d'Azur', flowers provided by Zwetsloots & Sons Ltd (UK) were originally purchased from two major freesia growers in The Netherlands (J. Kuyvenhoven, Naaldwijk, and Arie Stolk at LE De Lier). These flowers were processed in the laboratory approximately 24h after harvest (D. Zwetsloot pers. com., 2000) and were at the commercial bud stage (Appendix, Plate A2.2).

3.2.2.2 Postharvest flower inoculation

A single-spore *B. cinerea* Pers. isolate (BcF1) was used in the experiment (Appendix, 2.3.1). *B. cinerea* conidial suspensions were prepared by flooding the Petri plates (½ strength PDA, OXOID Ltd. Basingstoke, Hampshire, UK. 19.5 g L^{-1}) with sterile distilled water containing 0.05% Tween 80 (Sigma, St. Louis, USA) (Dhingra and Sinclair, 1995). Conidia were dislodged by gently rubbing the surface of the fungal colony with a sterile razor blade (Dhingra and Sinclair, 1995). The conidial suspension was filtered through two layers of sterile cheesecloth to remove mycelia. The final concentration of the conidial suspension was adjusted to 10^2, 10^3 or 10^4 conidia mL^{-1} with the aid of a haematocytometer (two counts per conidial suspension) (Marois *et al.*, 1988; Hammer and Evensen, 1994). The experimental freesia flowers were stood in 284 mL polystyrene containers containing tap water. Inoculation was by misting the flowers with the conidial suspensions by using a 2.5 L hand pump (Hozelock Ltd., Haddenham, Bucks, UK) until incipient run off (Dhingra and Sinclair, 1995).

3.2.2.3 Post-inoculation conditions

Inoculated freesia flowers were either covered with plastic transparent bags (12 cm x 16 cm) to maintain RH at *ca.* 100% (condensation was observed as free water on the flower petals) or left uncovered at RH of 80-90%. Humidity inside the incubation rooms was maintained at 80-90% with the aid of steam humidifiers (Bionaire C-11, Ambivent Ltd., Northampton, UK). In an attempt to cover common handling conditions (Figures 3.4-3.7), inoculated flowers were incubated at a range of temperatures. Flowers were initially incubated for 24h at 5, 12 and 20°C, which are temperatures usually occurring during handling process (Figures 3.4–3.7). Subsequently, they were incubated for additional 48h at 5°C, which was the temperature occurring inside storage rooms at Zwetsloots & Sons Ltd (D. Zwetsloot pers. comm., 2000).

3.2.2.4 Assessments

Disease

Disease assessments were carried out 24, 48 and 72h after artificial inoculation. The diameters of 10 randomly selected lesions per flower were measured under a x2 magnifier using a hand-held digital micrometer (stock No. 600-880, Mitutoyo, Japan). Disease severity was evaluated using the following arbitrary scale: 0 = no spots on the petals, 1 = 1-5% petal coverage by spots (enough for commercial rejection), 2 = 5-25% petal coverage by spots, 3 = 25-50% petal coverage by spots, and, 4 = 50-100% petal coverage by spots (Appendix 2.5, Plate A2.3). Disease severity was assessed on the three outside petals and the mean score for each flower was calculated. In addition, the number of lesions on petals were also counted.

Flower development and senescence

Flower development and senescence were scored every day after inoculation using the scale: 0 = bud stage, 1 = open flower, 2 = slightly senescent flower, 3 = moderately senescent flower, 4 = severely senescent flower, and, 5 = dead flower.

3.2.2.5 Experimental design and statistical analysis

The experiment was arranged inside controlled temperature incubation rooms in a completely randomized block (CBR) design with the three factors of temperature (5, 12 and 20°C), RH (80-90% and 100%) and inoculum level (10^2, 10^3 and 10^4 *B. cinerea* conidia mL^{-1}). Incubation rooms were not replicated due to insufficient number of incubation cabinets. However, the CRB design was based on the assumption that conditions inside the incubation cabinet did not vary (C. Marshall, pers. comm., 2002). Ten individual replicate flowers were used for each treatment. Data were analysed using a randomised complete block design (i.e. factorial) ANOVA model (univariate ANOVA) to compare main factor means. Individual treatment means were compared using the Duncan's multiple range test at P = 0.05 (Field, 2000). The non-parametric Kruskal-Wallis test was used to determine differences in disease severity rating scoring within factors (Little, 1985). Correlations between senescence and disease variables (i.e. disease severity, lesion numbers and lesion diameters) were carried out using Pearson's correlation test. Statistical analysis was performed using SPSS 9.0 (Statistical Package for the Social Science, Chicago, IL, USA) for Windows. Linear regression analysis and graphic presentation was performed in Sigmaplot 2000 (Chicago, IL, USA) for Windows. Data in text are presented as main factor means in tables and the corresponding individual treatment means are presented in figures. The results of the statistical tests are presented in appendices.

3.2.2.6 Light microscopy

Freesia sample fixation

Whole freesia petals were fixed in formalin - acetic acid - ethanol (F.A.A) solution made of 5 mL formalin, 5 mL glacial acetic acid, and 90 mL 70% ethanol (v/v) (Purvis *et al.,* 1966). The solution was made immediately before use to avoid chemical reaction, which would have resulted in unstable fixative.

Sample dehydration and clearing

Fixed freesia pieces were dehydrated, cleared and infiltrated in the series of ethanol, toluene and wax immersions as set out in Table 3.1 (Purvis *et al.,* 1966).

Table 3.1: Steps for freesia sample dehydration and clearing

Step	Solvent (v/v)	Time
1	70% ethanol	3 h
2	80% ethanol	1 ½h
3	90% ethanol	1 ½h
4	100% ethanol	1 ½h
5	100% ethanol	1 ½h
6	toluene	1 ½h
7	toluene	1 ½h
8	parrafin wax	1 ½h
9	parrafin wax	1 ½h
10	parrafin wax	½h

Embedding, making the block and sectioning

After sample infiltration with wax the tissue was cast into a block of fresh wax. 10 µm thick sections were cut with a microtome carrying a steel knife (Bright Instrument

Co. Ltd., Huntingdon, UK) and floated-out on warm water (*ca.* 50°C) in which gelatin (0.1% v/v) had been dissolved to act as an adhesive.

Section staining

Sections were stained with Safranin O and Fast Green FCF for lignin, cutin, suberin and chitin staining (Ruzin, 1999). The following method was used: sections were first de-waxed, washed with distilled water, stained in Safranin for 24h and then washed in tap water to remove excess stain. They were dehydrated in 50% and 70% (v/v) ethanol for 2-5 min per step. They were then counterstained in Fast Green for 10 sec, dipped rapidly through an ethanol (70, 80, 90, 100, and 100% v/v) series to remove traces of water, cleared in toluene and finally mounted in DPX. Samples were examined under a Leitz Laborlux K compound microscope fitted with Ploempak incident light fluorescence illuminator with filter blocks I_2 (exciting filter 450-490, beam splitter 510, barrier filter 515 and exciting filter 340-380, Beam splitter 400, barrier filter 430) (Leica Microsystems, Milton Keynes, UK). All samples were examined under fluorescent or cool light.

For visualisation of infection, freesia petals or petal sections were fixed in methanol-glacial acetic acid (1:1, v/v) for 48h, cleared with 75% lactic acid for 48h at 40°C and stained in lactophenol aniline-blue solution (Erb *et al.*, 1973) (Appendix 2.1, A2.1.2.4). Stained material was transferred into lactophenol to remove excess stain and mounted on glass slides. After 1 h of incubation at room temperature samples were examined under fluorescent or cool white light.

3.2.3 Results

Temperature, RH and inoculum level had a marked significant effects ($P < 0.05$) on disease severity and lesion number on freesia var. 'Cote d' Azur' flowers inoculated with *B. cinerea* (Table 3.2, Appendix 3.2, Tables A3.2.4 and A3.2.6). Inoculation of freesia flowers with 10^4 *B. cinerea* conidia mL^{-1} gave significantly ($P < 0.05$) higher disease severity and lesion numbers compared to flowers inoculated with 10^2 and 10^3

conidia mL^{-1} (Table 3.2). However, flowers inoculated with 10^3 conidia mL^{-1} did not differ significantly ($P > 0.05$) in disease severity, lesion numbers or lesion diameters from those inoculated with 10^2 conidia mL^{-1} (Table 3.2). Inoculum level did not significantly ($P > 0.05$) affect lesion diameter (Table 3.2). Incubation of freesia flowers for 72h at 12 or 20°C resulted in significantly ($P < 0.05$) higher disease severity scores and lesion numbers compared to flowers incubated at 5°C for 72h (Table 3.2). In contrast, lesion diameter was significantly ($P < 0.05$) higher on flowers incubated at 5°C (Table 3.2). Incubation of freesia flowers at 100% RH also resulted in a significant ($P < 0.05$) increase in disease severity and lesion numbers compared to flowers incubated at 80-90% RH (Table 3.2). At 5°C and 100% RH an abrupt increase in disease severity, lesion number and lesion diameter on freesia petals was observed from 24 to 48h of incubation.

Disease severity, lesion numbers and lesion diameters on freesia flowers inoculated with *B. cinerea* and incubated at 100% RH increased over time (Figures 3.6, 3.7 and 3.8). In contrast, lesion numbers and lesion diameters on freesia flowers incubated at 80-90% RH did not change over time.

Table 3.2: Effects of inoculum level, temperature and RH on disease severity, lesion number and lesion diameter on freesia var. 'Cote d' Azur' flowers. Disease assessments were carried out every day after artificial inoculation. Data for independent treatment means are presented in Figures 3.6. 3.7 and 3.8..

Factors	**Variables**		
	Disease severity (score 0-4) [a]	**Lesion number**	**Lesion diameter (mm)**
1) Inoculum level (*B. cinerea* conidia mL^{-1})			
10^2	0.7 a	21 a	0.8 a
10^3	0.8 a	23 a	0.7 a
10^4	1.4 b	44 b	0.8 a
2) Temperature (°C)			
5 - 5	0.7 a	20 a	0.9 a
12 - 5	1.2 b	36 b	0.7 b
20 - 5	1.1 b	32 b	0.7 b
3) Relative humidity (RH)			
80-90% (Unsleeved)	0.2 b	6 a	- [b]
100% (Sleeved)	1.8 a	53 b	0.8
4) Time (hours)			
24	0.7 a	21 a	0.6 a
48	1.0 b	31 b	0.7 b
72	1.3 c	36 c	0.9 c

[a] Within main factor means, numbers followed by the same letter are not significantly different at P = 0.05

[b] Lesion diameter of flowers incubated at 80–90% RH was not measured.

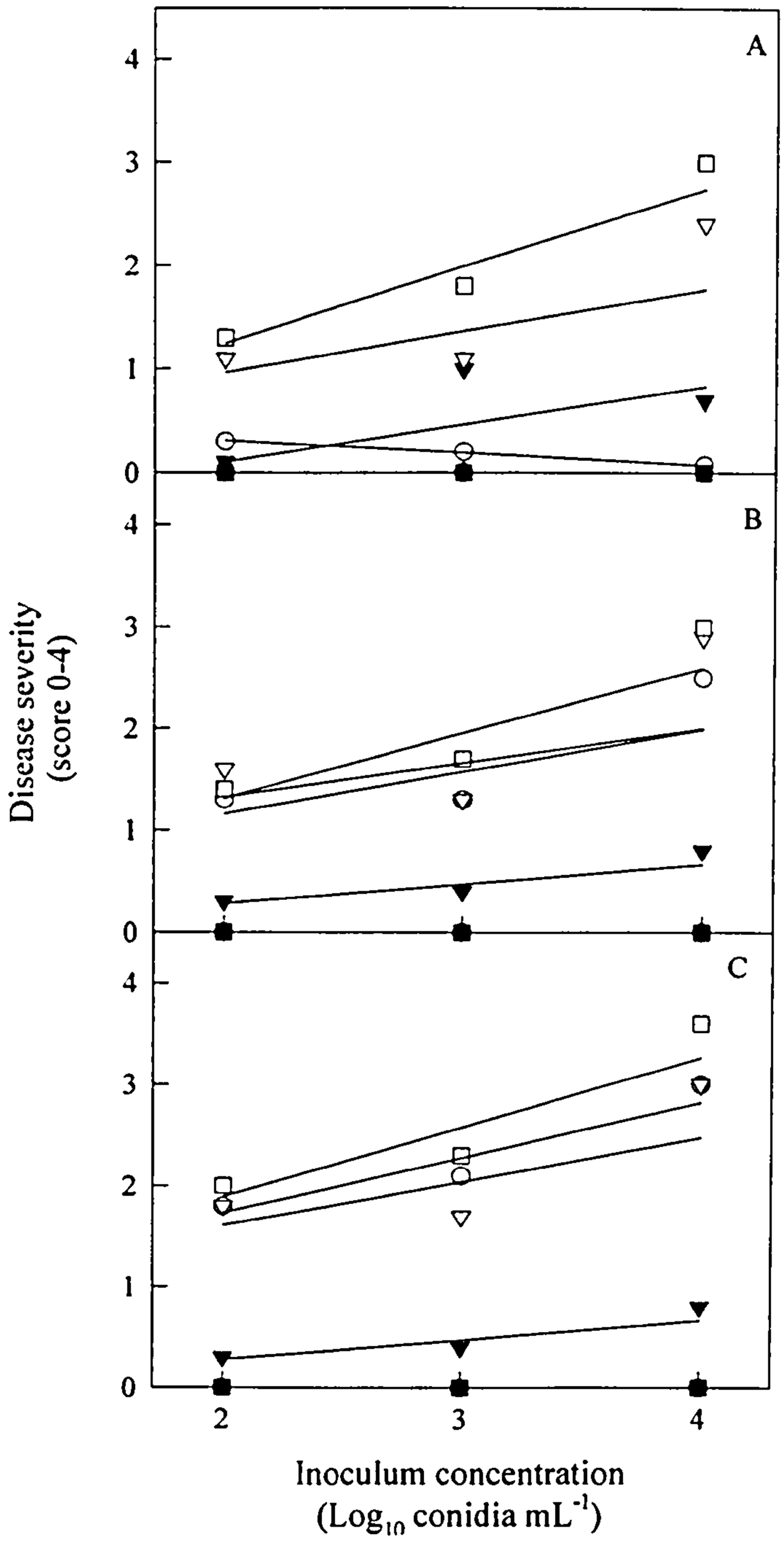

Figure 3.6: Disease severity on freesia var. 'Cote d'Azur' flowers inoculated with 10^2, 10^3 and 10^4 *B. cinerea* conidia mL^{-1}. Flowers were initially incubated for 24h at 5°C and 100% RH (○), 5°C and 80-90% RH (●), at 12°C and 100% RH (▽), 12°C and 80-90% (▼), 20°C and 100% RH (□) and at 20°C and 80-90% (■) (A). All flowers were then transferred at 5°C for 48h (B) and 72h (C) at 80–90 or 100% RH. Data are means of 10 replication flowers. Corresponding estimated parameters are presented in Appendix 3.2, Table A3.2.5.

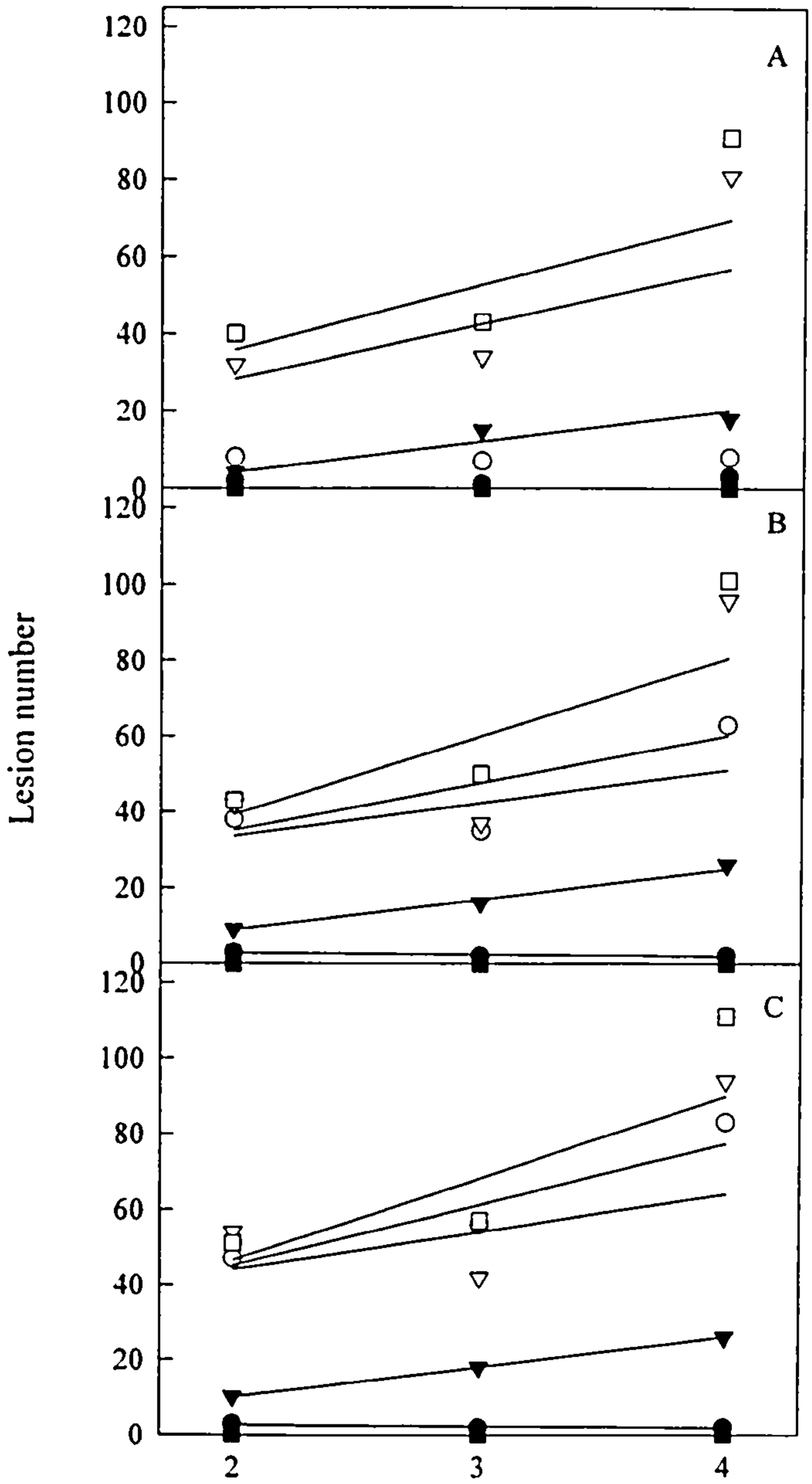

Figure 3.7: Lesion number of freesia var. 'Cote d'Azur' flowers inoculated with 10^2 10^3 and 10^4 *B. cinerea* conidia mL^{-1}. Flowers were incubated initially for 24h at 5°C and 100% RH (○), 5°C and 80-90% RH (●), at 12°C and 100% RH (▽), 12°C and 80-90% (▼), 20°C and 100% RH (□) and at 20°C and 80-90% (■) (A). All flowers were then transferred at 5°C for 48h (B) and 72h (C) at 80–90 or 100% RH. Data are means of 10 replication flowers. Corresponding estimated parameters are presented in Appendix 3.2, Table A3.2.7.

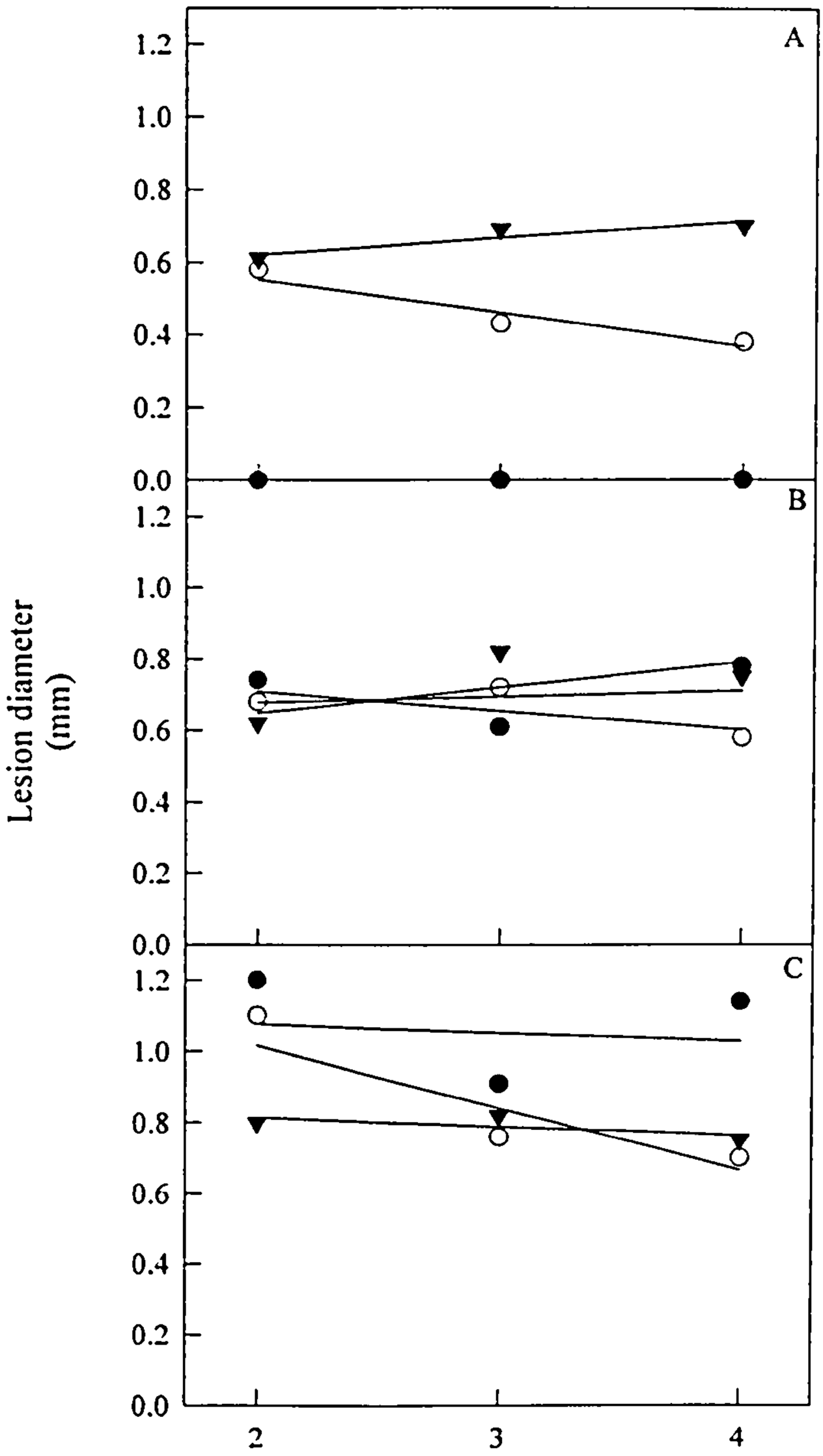

Figure 3.8: Lesion diameter of freesia var. 'Cote d'Azur' flowers inoculated with 10^2 10^3 and 10^4 *B. cinerea* conidia mL^{-1}. Flowers were incubated initially for 24h at 5°C and 100% RH (●), at 12°C and 100% RH (○) and at 20°C and 100% RH (▼) (A). All flowers were then transferred at 5°C for 48h (B) and 72h (C) at 100% RH. Data are means of 10 replication flowers. Corresponding estimated parameters are presented in Appendix 3.2, Table A3.2.9.

Disease severity, lesion number and lesion diameter was constantly at zero levels on flowers incubated at 20°C and 80-90% and negligible on flowers incubated at 5°C and 80-90% RH (Figures 3.6, 3.7 and 3.8 A, B, C). Nevertheless, lesion number means per flower incubated at 5°C and 80-90% RH would have resulted in flower rejection by flower importers (see section 3.2.1.4). In all cases, disease severity and lesion numbers increased with increasing inoculum level independent of incubation temperature (Figures 3.6, 3.7 and 3.8 A, B, C). However, only in one case (flowers incubated for 24h at 5°C and 100% RH) was the correlation between incubation conditions and disease severity significant ($R^2 = 0.99$, $P = 0.04$) (Table 3.4). In contrast, lesion diameters on freesia petals were not affected by inoculum level but were temperature dependent (Figure 3.8 A, B, C).

Senescence of freesia flowers after 72h of incubation was significantly ($P < 0.05$) positively correlated with disease severity and lesion numbers (Table 3.3). However, senescence was not significantly ($P > 0.05$), correlated with lesion diameters (Table 3.3).

Table 3.3: Effect of senescence on disease severity, lesion numbers and lesion diameters on freesia var. "Cote d' Azur" flowers inoculated with *B. cinerea*. Data are results from Pearson's correlation.

Disease variables	**N** [a]	**Senescence** [b]
Disease severity	540	0.404 **
Lesion number	540	0.455 **
Lesion diameter	240	-0.053 ns

[a] Number of observations
[b] Data are results from Pearson's correlation at P = 0.05
** Significant at P = 0.01, ns: not significant at P = 0.05

B. cinerea apparently infected freesia petal epidermal cells by direct penetration without forming appressoria (Plate 3.4 C). Conidium were shown to be able to produce two infection pegs. In some cases germinating conidia failed to penetrate epidermal cells (Plate 3.4 A, B). After infection, advanced lesion development produced a brown, volcano shaped lesion (Plate 3.5).

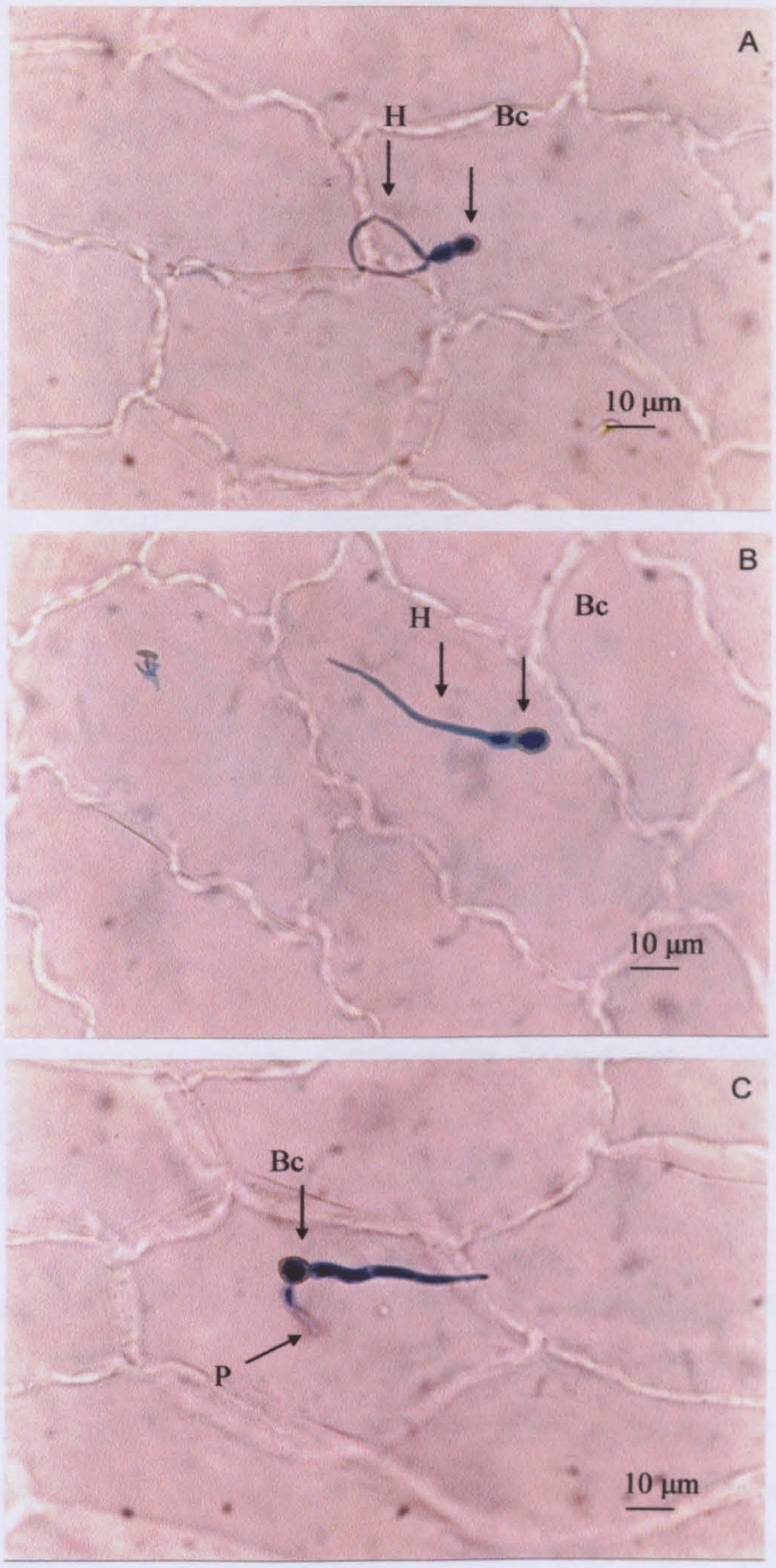

Plate 3.4: Examples of different germinated *B. cinerea* conidium (A, B, C) on the surface of artificially inoculated freesia petals incubated at 100% RH for 3 days at 5°C. The conidium has apparently penetrated petal surface without forming an appressorium structure (C). Samples were prepared as described by Erb *et al.* (1973). Bc: *B. cinerea* conidium, H: Hyphae, P: Probable penetration site.

Plate 3.5: Extended necrotic lesions (arrows) on freesia var. 'Cote d'Azur' flowers artificially inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 4 days. Lesions were brown in colour and had a volcano-like shape.

Lesions expanded differently at low versus high incubation temperatures. At 5°C, *B. cinerea* lesions on freesia petal surface developed slower than at 20°C. Thereafter, lesion diameter in flowers incubated at 20°C did not increase much even after 72h of incubation. Production of lignin and/or callose on freesia petals was observed after infection by *B. cinerea* at both incubation temperatures tested (Plate 3.6 and 3.7). Infection proceeded only on the first 2-3 layers of the outer surface of the petal (Plate 3.8 and 3.9). Infection of the adaxial flower surface was also observed.

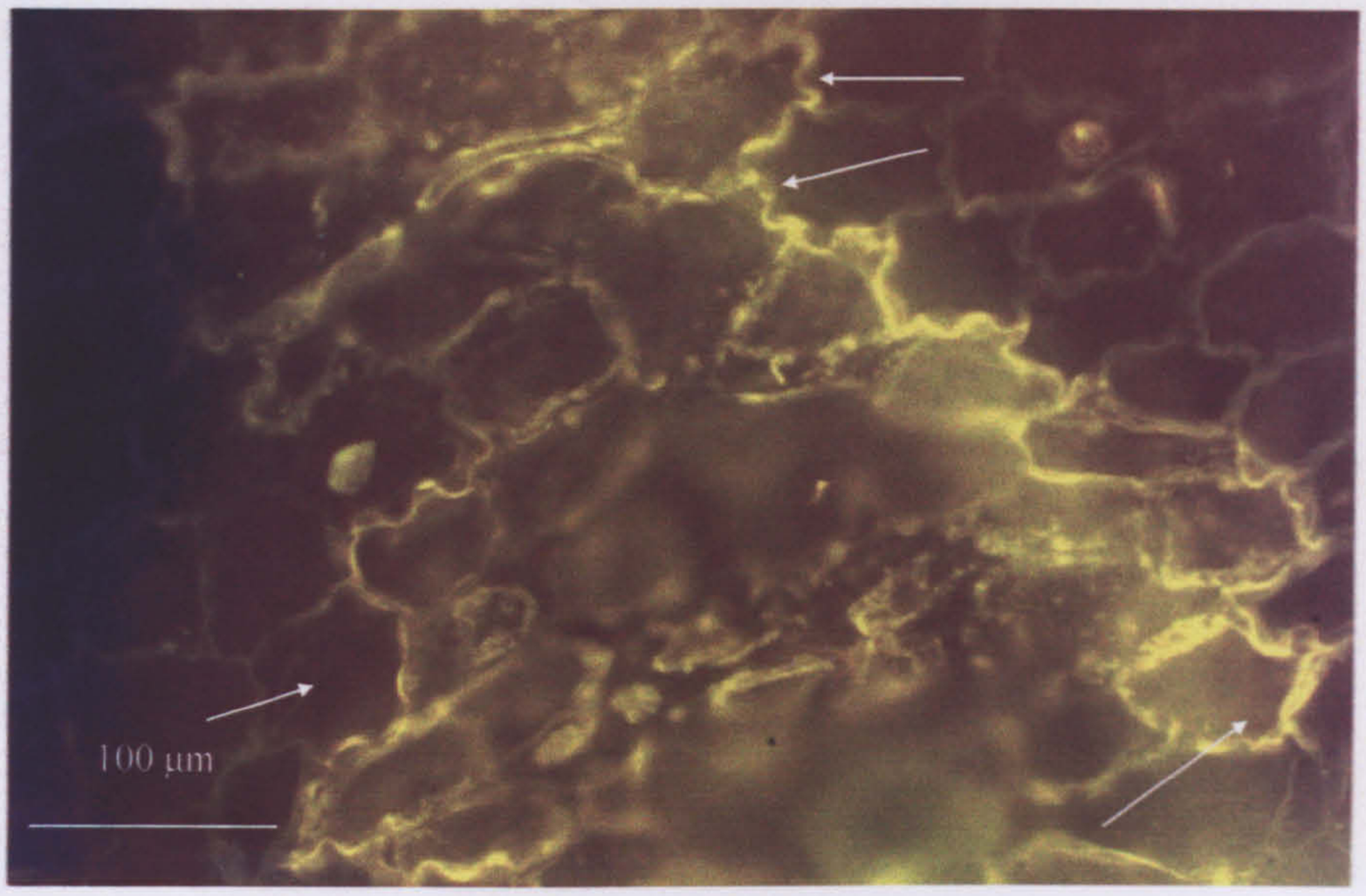

Plate 3.6: Freesia petal cells macerated by *B. cinerea* hyphae at 5°C. The fungus had colonized approximately 40 adjacent epidermal cells after 4 days of incubation at 5°C. Fluorescing tissue under UV-light at 354 nm (arrows) indictates deposition of lignin and callose (Ruzin, 1999).

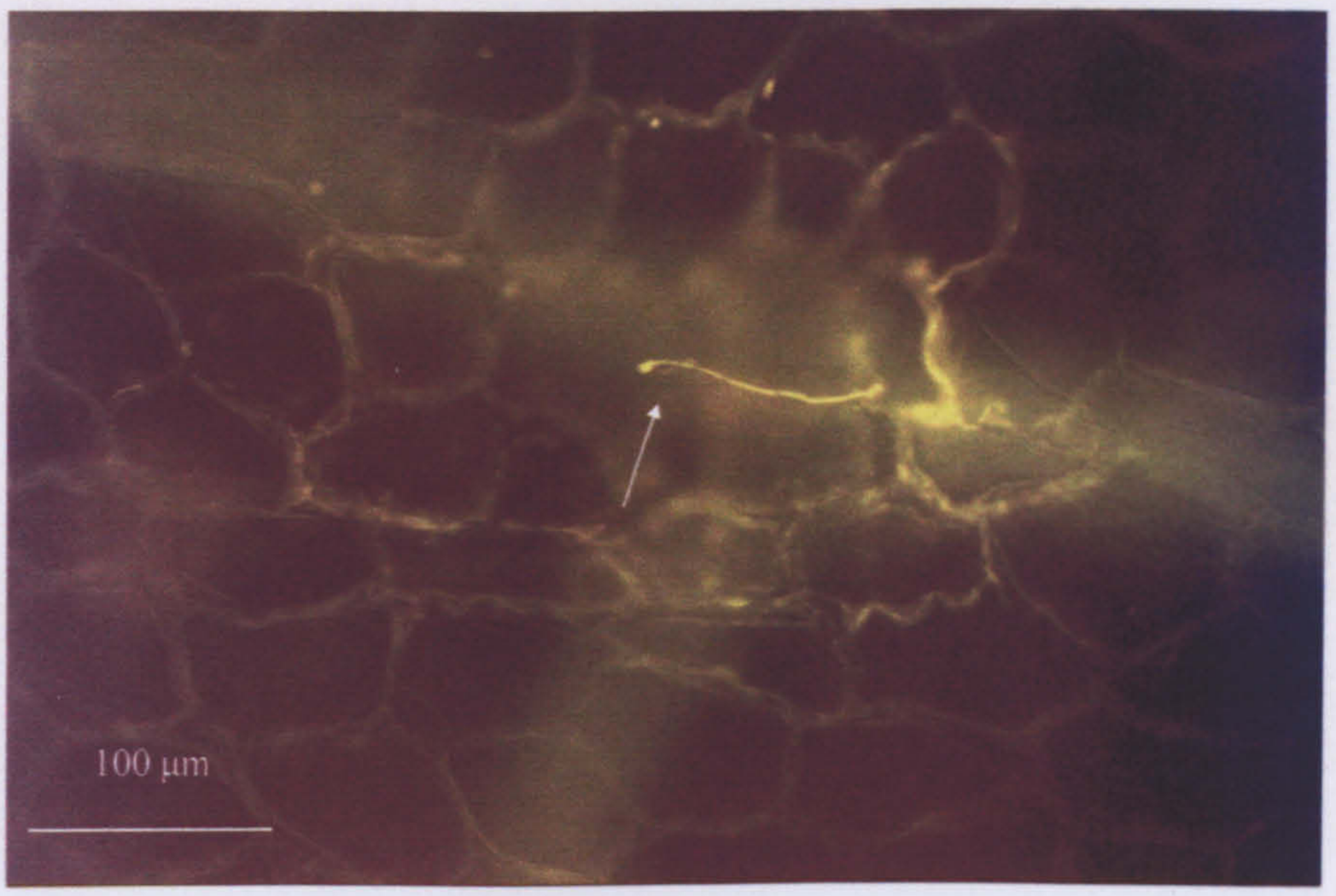

Plate 3.7: Freesia petal cells macerated by *B. cinerea* hyphae at 20°C. The fungus had colonized approximately 20 adjacent epidermal cells after 24h of incubation at 20°C. Fluorescing tissue under UV-light at 354 nm (arrows) indictates deposition of lignin and/or callose (Ruzin, 1999). Arrow indicates *B. cinerea* germinated conidium which possibly produced the lesion.

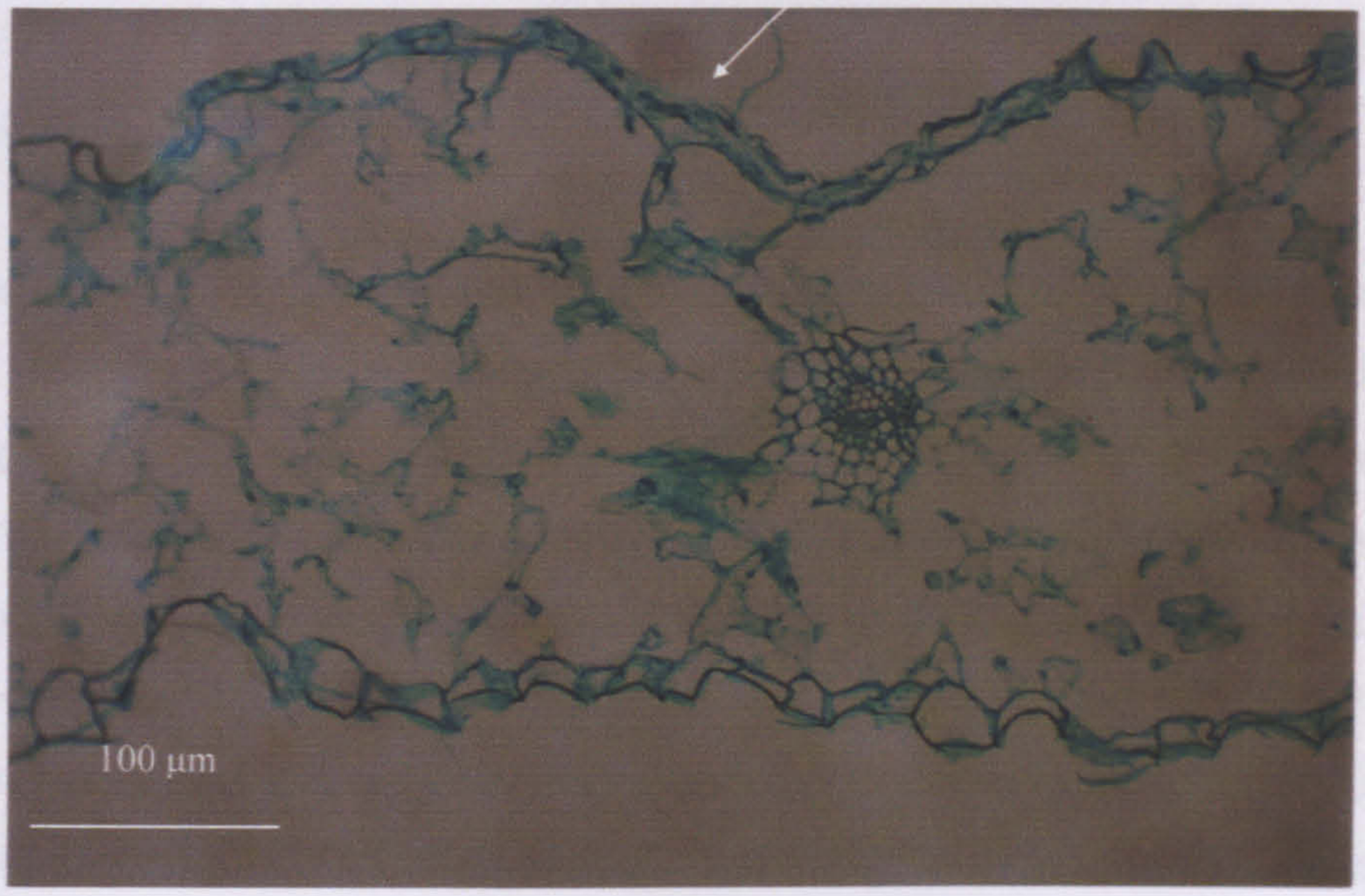

Plate 3.8: Cross-section of lesion produced by *B. cinerea* on freesia petals (arrow). Lesion had a volcano-like shape (arrow).

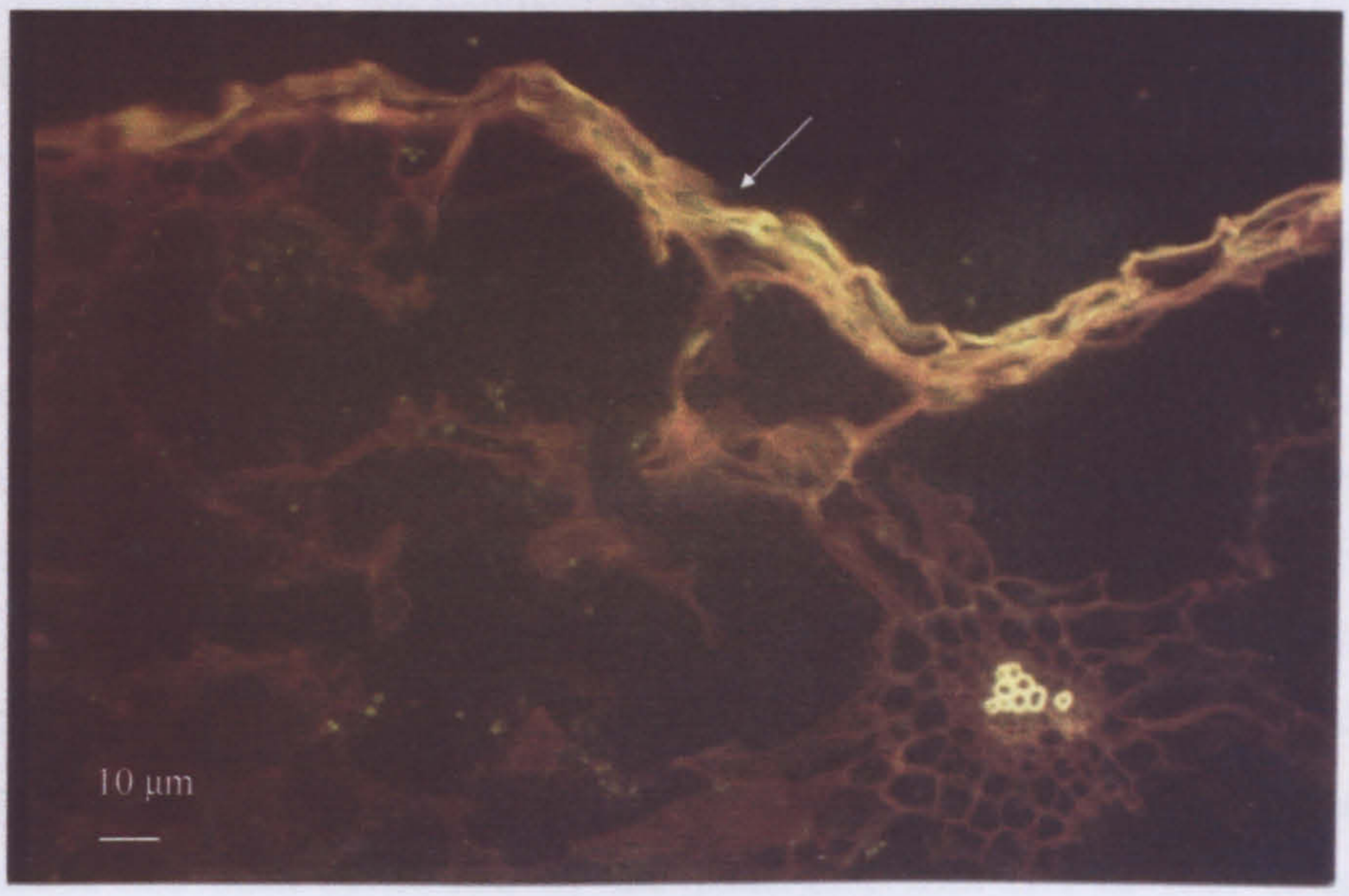

Plate 3.9: Cross-section of lesion produced by *B. cinerea* on freesia petals (arrow). This is the same picture as above (Plate 3.8) with the lesion site fluorescing under UV-light at 354 nm (arrow) due to lignin and callose deposition (Ruzin, 1999).

3.2.4 Discussion

The results of the present study show that in the presence of *B. cinerea* inoculum on freesia petal surface temperature was not a limiting factor for disease establishment. According to Salinas *et al.* (1989), germination of *B. cinerea* conidia and lesion formation on gerbera flowers occurred over the wide range of temperatures from 4-25°C. Incubation of artificially inoculated freesia flowers at 12°C resulted in overall higher disease severity and lesion numbers compared to flowers incubated at 5 or 20°C. This observation contradicts earlier studies suggesting 20°C as the optimum temperature for *B. cinerea* infection (Adair, 1971; Bulger *et al.,* 1987; Salinas *et al.,* 1989; Nair and Allen, 1993; Kerssies, 1994). However, in practice vegetable and flower growers in Greece are often advised to increase temperature levels to 18–20°C inside glasshouses to stop *B. cinerea* infection spread (Dr I. Vloutoglou, Benaki Phytopathological Institute, Athens, Greece, pers. comm., 2003).

Incubation duration substantially affected *B. cinerea* development on freesia flowers. Disease severity, lesion number and lesion diameter at 5°C was limited after 24h of incubation. Thereafter, a sudden increase in disease severity, lesion numbers and lesion diameters after 48 and 72h of incubation was recorded. In gerberas, petal colonization by *B. cinerea* at 4°C and 100% RH was observed after 18 days of incubation (Salinas and Verhoeff, 1995). Incubation of inoculated freesia flowers at 20°C resulted in the formation of restricted lesions, which did not markedly expand over time. The necrotic lesions produced at 20°C by *B. cinerea* on freesia petals, had a volcano-like shape and were similar to those reported on rose by Pie and De Leeuw (1991) and on gerberas (Salinas and Verhoeff, 1995). Salinas and Verhoeff (1995) reported that at temperatures between 18 and 25°C, *B. cinerea* growth usually stopped soon after the gerbera cuticle had been penetrated by the pathogen, with two to four cells around penetration site becoming necrotic. However, when gerbera flowers were incubated at 4°C, lesions became visible after 3 days of incubation and consisted of a group of 10-14 necrotic cells. Thereafter, *B. cinerea* infection affected 40-50 necrotic cells after 18 days of incubation at 4°C (Salinas and Verhoeff, 1995).

The present study also showed that relative humidity (RH) was the most crucial factor for infection of freesia flowers by *B. cinerea*. The environmental conditions during the first 24h after inoculation were critical for *B. cinerea* disease establishment. Reducing RH inside incubation rooms and/or eliminating petal wetness resulted in suppression of *B. cinerea* disease independent of the temperature and inoculum level used. At 5°C and 80-90% RH, visible *B. cinerea* lesions were recorded after 48h of incubation. In contrast, at 12°C and 80-90% RH, *B. cinerea* produced visible necrotic lesions just after 24h of incubation. Incubation of freesia flowers at 20°C and 80-90% RH did not produce visible lesions even after 72h of incubation. Williamson *et al.* (1995) reported that infection of rose petals by *B. cinerea* failed at RHs below 94%. Salinas *et al.* (1989) also reported that at temperatures ranging from 18-25°C and 100% RH for at least the first 5h after inoculation was necessary for *B. cinerea* establishment in gerberas. Lower RH regimes resulted in failure of *B. cinerea* conidia to germinate and infect gerberas (Salinas *et al.*, 1989; Salinas and Verhoeff, 1995). Under continuous wetness, 50% of grape flowers and berries were infected by *B. cinerea* in less than 6h at temperatures ranging from 10 to 30°C (Nair and Allen, 1993).

Overall, inoculum level markedly affected disease severity and lesion numbers formed on freesia petals. Disease severity and lesion numbers increased linearly with inoculum dose. These results are in agreement with those by Marois *et al.* (1988), who reported that there was a linear relationship between *B. cinerea* inoculum concentration and disease severity on rose flowers.

3.4 GENERAL CONCLUSIONS

Freesia stem rejections due to *B. cinerea* infection varied during the year 2000. The total number of rejected freesia stems peaked in May, August and October 2000. Pre-harvest temperatures ranging from 10-17°C were associated with higher proportions of rejected freesia stems. However, there was no linear or quadratic correlation between environmental conditions inside or outside the glasshouse in The Netherlands and rejections in the UK.

The results of the present study also showed that the time needed for freesia petals to become infected by *B. cinerea* was less than 24h at 12°C and 80-90% RH. Such conditions commonly occur during freesia transportation. The time taken for freesia flowers to be transported from The Netherlands to the UK is typically sufficient for infection of both gerbera (Salinas *et al.* 1989) and freesia flowers. Therefore, the development of *B. cinerea* lesions on freesia flower petals could conceivably occur during the first 24 to 65h of postharvest handling in transit from flower auction in The Netherlands to the wholesaler in the UK. This scenario is especially likely under RH levels ranging between 80-100% or in the presence of a water film on petal surfaces and at temperatures ranging from 4-25°C. At saturated RH (100%) disease symptoms were visible within only 24h of constant temperature of 5°C. Increases in temperatures resulted in faster disease development. RH regimes rarely reached levels above 95% during handling chain, but temperature fluctuations could easily result in water condensation on freesia petals and thereby facilitate infection by *B. cinerea*. In the present study, temperature rarely reached the ideal low storage temperatures for freesia flowers (Goszczynska and Rudnicki, 1988). Rather, temperatures during transportation ranged from 8-16°C. Precise control of temperature during transportation is crucial to avoid rapid *B. cinerea* development (Salinas and Verhoeff, 1995). However, as temperature is not a completely limiting factor for *B. cinerea* development (Salinas *et al.*, 1989), *B. cinerea* disease suppression might best be achieved by protective pre- or post-harvest chemical or biological treatments. Novel chemical or biological treatments that would increase flower resistance to postharvest infection by *B. cinerea*. Infection may be suppressed if a stronger host hypersensitive reaction was induced. This may restrict pathogen development in smaller areas inside freesia tissue. It follows that, visible lesions would be reduced and therefore rejections avoided.

CHAPTER 4

NOVEL POSTHARVEST TREATMENTS TO CONTROL SPECKING OF CUT FREESIA FLOWERS CAUSED BY *BOTRYTIS CINEREA*

4.1 SUPPRESSION USING POSTHARVEST TREATMENT WITH ACIBENZOLAR-S-METHYL

4.1.1 Introduction

Control of *Botrytis* disease can be achieved by pre- and/or postharvest chemical applications. For example, postharvest infection by *B. cinerea* on table grapes was suppressed with sulfur dioxide (SO_2) treatments and rapid pre-cooling during storage or transportation (Nelson, 1991). However, if the cool handling chain is not maintained properly, excessive SO_2 may be released and cause phytotoxic symptoms and sulfurous flavour to the berries (Winkler *et al.,* 1974). Furthermore, control of *B. cinerea* with conventional fungicides carries the risk of encouraging development of fungicide resistant strains. Many cases of *B. cinerea* resistance to iprodione (Rovral) and benomyl (Benlate) have been reported (e.g. Joyce and Wearing, 1996). Additionally, there is increasing public concern over fungicide usage in terms of human and environmental risk (Jacobsen and Backman, 1993). Novel integrated pesticide management (IPM) strategies have been proposed to suppress various fungal pathogens on different crops and to minimise the risk of resistance development by pathogens (Jacobsen and Backman, 1993; Kessmann *et al.,* 1994; Lucas, 1999).

Activators of plant defence reactions such as acibenzolar-S-methyl can reduce certain diseases by enhancing natural defence mechanisms (Kessmann *et al.,* 1994; Lucas, 1999) (Table 2.5). In many cases, systemic protection of crops by acibenzolar and other plant activators has been achieved with applications made at various intervals before harvest (Kessmann *et al.,* 1994) (Table 2.5). Induction of systemic acquired resistance (SAR) can facilitate a relative long lasting resistance against a broad range of

pathogens (Kessmann *et al.,* 1994). Therefore, SAR could be a useful tool in IPM strategies. The residual efficacy of acibenzolar has been tested postharvest on crops after preharvest applications (e.g. Huang *et al.,* 2000; Terry and Joyce, 2000). No published evidence was found on whether acibenzolar has been tested as postharvest treatments on ornamental crops. However, due to the economic importance of freesia crops, novel ways for controlling postharvest infection by *B. cinerea* and associated merchandise losses are required.

This study investigated the efficacy of postharvest treatment of freesia flowers with acibenzolar in suppressing *B. cinerea* through induction of SAR. This plant activator acibenzolar was tested under a range of temperatures and varying inoculum levels. Possible direct antifungal activity of acibenzolar was also tested *in-vitro.* Phenylalanine ammonia lyase (PAL) assays of freesia flowers were performed to investigate possible secondary metabolite induction by acibenzolar treatment. PAL is an important enzyme in pathogen growth restriction as it regulates the production of phenylpropanoid derivatives, many of which have specific antifungal properties (Ebel, 1986; Kombrink and Somssich, 1995; Kuć, 1995). In the present study, the influence of acibenzolar on freesia vase life was also ascertained.

4.1.2 Materials and methods

4.1.2.1 Plant material

Cut freesia flowers of vars. 'Cote d' Azur' and 'Texel' were provided by Zwetsloots & Sons Ltd (UK) (Appendix 2.2, Plate A2.1). These flowers were at the commercial stage with all buds still closed (Appendix 2.2, Plate A2.2). They were processed in the laboratory approximately 24h after harvest. Freesia flowers of var. 'Texel' were used in two acibenzolar experiments, one for disease assessments and one for phenylalanine ammonia lyase (PAL) enzyme activity assays.

4.1.2.2 Experiment design

These experiments were conducted in CRB designs with 4-7 blocks and 4-16 flowers in each block depending on the experiment (Table 4.1). Experiments A1, A2 were arranged inside controlled temperature incubation rooms in a CRB design. These experiments were two-factor designs with temperature (5, 12 and 20°C) and acibenzolar treatment (0, 0.15, 0.3 and 0.6 g AI L^{-1}) as factors. Experiment A3 was also arranged inside controlled temperature incubation rooms in a CRB design. This experiment was a three-factor design with temperature (5, 12 and 20°C), chemical treatments (untreated, acibenzolar, iprodione and acibenzolar+iprodione) and inoculum level (0, 10^3, 10^4 and 10^5 *B. cinerea* conidia mL^{-1}) as factors. Experiment A4 was arranged in a completely randomised (CR) design in a vase life room with acibenzolar treatment (0, 0.15, 0.3 and 0.6 g AI L^{-1}) as factor.

Table 4.1: Experiment number, freesia variety, number of replications, inoculum treatment concentration, assessment parameters and other treatment variables pertaining in the acibenzolar experiments.

Experiment	**Variety**	**Replications**	**Inoculum concentration (No. of conidia mL^{-1})**	**Assessments** [a]	**Factors** [b]
A1	Cote d'Azur	5	10^4	Disease	T x A
A2	Cote d'Azur	5	-	Disease	T x A
A3	Texel	4	0, 10^3, 10^4, 10^5	Disease	T x A x I
A4	Cote d'Azur	7	-	Vase life	A
A5	Texel	4	-	PAL assay	Ti x A
A6	*In-vitro*	5	2 x 10^6		A

[a] Disease assessments include disease severity, lesion numbers and lesion diameter.
[b] T: Temperature, A: Acibenzolar treatment, I: Inoculum level, Ti: Time.

4.1.2.3 Acibenzolar-S-methyl treatments

For acibenzolar-S-methyl treatments in experiments A1, A2, and A4, acibenzolar (Bion®, 50% wettable granulated formulation, UK) was provided by Novartis Crop Protection (Cambridge, UK). Acibenzolar was used at 0 (control) and three concentrations of 0.15, 0.3 and 0.6 g AI L^{-1}. The concentration 0.15 g AI L^{-1} is the recommended dose for monocots by Novartis (Pat Ryan, pers. comm., 2000). In experiment A3, flowers were sprayed with either acibenzolar at 0.15 g AI L^{-1} or iprodione (Rovral, 50% w.p., Aventis, UK) at 0.5 g AI L^{-1}. The concentration of 0.5 g iprodione L^{-1} being the recommended dose for ornamentals. A mixture of acibenzolar and iprodione at 0.15 g AI L^{-1} plus 0.5 g AI L^{-1}, respectively was also used. In experiment A5, flowers were sprayed with acibenzolar at 0.15 g AI L^{-1}.

Chemical solutions were prepared by dissolving the chemicals in distilled water. Freesia flowers were sprayed with a 2.5 L capacity hand sprayer until incipient run-off (Terry and Joyce, 2000). Sprayed flower stems were placed into 284 mL vases and kept at 20°C (Thomma *et al.*, 2000). Twenty-four hours later, flowers were inoculated with the designated *B. cinerea* conidial suspensions and then covered with transparent plastic bags to maintain high (*ca.* 100%) RH. These artificially inoculated flowers were incubated at 5, 12 or 20°C in the dark.

4.1.2.4 Artificial inoculation

B. cinerea conidial suspensions were prepared as described in section 3.2.2.2. They were adjusted to 0, 10^3, 10^4 or 10^5 *B. cinerea* conidia mL^{-1} depending on experiment (Marois *et al.*, 1988; Hammer and Evensen, 1994).

4.1.2.5 Assessments

Disease

In experiments A1, A2 and A3, disease severity, lesion numbers, lesion diameters and senescence were recorded on the artificially inoculated flowers. Only the number of lesions were recorded on non-inoculated flowers. Disease severity on artificially inoculated flowers was evaluated daily using the arbitrary scale described previously in section 3.2.2.4 (Appendix 2.5, Plate A2.3).

Flower development and senescence

Flower development and senescence were also scored every day following inoculation using the scale described in section 3.2.2.4.

Vase life

In experiment A4, vase life (in days), flower fresh weight and wilt score were recorded. Flower fresh weight was measured every second day with a digital balance (Sauter RE2021, Albstadt - Ebingen, Germany). Data are presented as proportional (%) change relative to the initial fresh weight. The mean wilt score was recorded daily on all first four flowers of the spike (n = 4) using the arbitrary flower development and senescence scale described above.

Detached petal bioassays

Lesion diameters on detached freesia petals were recorded in experiment A1 according to the method of Meir *et al.* (1998). Freesia petals were detached from flower stems and placed inside a sealed transparent plastic box on top of a plastic net tray. Chemical treated flowers for 0 (control), 0.15, 0.3 and 0.6 g AI of acibenzolar L^{-1} were arranged in a completely randomized design inside the sealed box. To maintain *ca.* 100%

RH, a wet paper tissue was placed underneath the plastic net. The petals were wounded with a sterile biological needle and a 10 μL drop of *B. cinerea* conidial suspension of 10^6 conidia mL^{-1} (approximately 10 conidia per drop) was placed on the wound site. Lesion diameter was measured as described in section 4.1.2.5.

4.1.2.6 *In-vitro* acibenzolar activity

B. cinerea *mycelial growth*

The effects of sterile solutions of acibenzolar-S-methyl on *B. cinerea* mycelial growth were tested as described by Terry and Joyce (2000). Acibenzolar was added to warm (*ca.* 40°C) ½ strength PDA and poured into 9 cm Petri dishes. Acibenzolar was tested at concentrations of 0.15, 0.3 and 0.6 g AI L^{-1}. Petri plates were inoculated by placing a 0.5 cm diameter mycelium plug of a 12-day-old *B. cinerea* isolate (BcF1) at the centre of each plate. The cultures were then incubated at 20°C in the dark. Two diameters at right angles of the fungal colonies were measured every day after inoculation over a period of 5 - 6 days.

B. cinerea *conidial germination and germ tube elongation*

Potentially direct antifungal effects of acibenzolar-S-methyl were also tested on *B. cinerea* conidial germination and germ tube elongation. Acibenzolar solutions were prepared at concentrations of 0 (control), 0.15, 0.3 and 0.6 g AI L^{-1}. A *B. cinerea* conidial suspension of 2×10^6 conidia mL^{-1} was prepared in 0.5% sucrose (Adikaram *et al.*, 2002) and a 10 μL drop of this suspension was placed on glass slides previously cleaned with ethanol. An additional 10 μL drop of chemical solution was placed on top of the *B. cinerea* spore suspension drop. Two 20 μL drops per slide and two slides per chemical treatment were examined. Plastic Petri dishes were used as incubation chambers. Wetted Whatman No. 4 filter paper was placed on the base (bottom) of the dish and a supporting plastic net laid on top of it to avoid direct contact between the wet

paper below and the glass slide above. The Petri dishes with the glass slides were incubated for 12h at 20°C in the dark. Upon removal from the incubator, a 10 μL lactophenol drop was added to each glass slide to terminate further conidial germination and a cover slip was placed on top of the slide. Proportional (%) conidial germination and germ tube elongation for each treatment were determined on 100 conidia randomly selected in each drop. Germ tube elongation was measured under a microscope (Leica Microsystems, Milton Keynes, UK) equipped with a graticule at x16 magnification (Adikaram *et al.*, 2002).

4.1.2.7 Biochemical assays

In experiment A5, PAL activity was measured in selected treatments. Freesia flowers were snap frozen and grounded in liquid nitrogen and were added to 50 mL prechilled (–18°C) acetone. This slurry was then homogenized with an Ultra Turrax T 25 S7 homogeniser (Janke & Kunkel GMBH & Co. KG IKA Labotechnik) at 9000 rpm for 1 min. The supernatant was washed with 50 mL cold acetone and filtered through a Whatman No. 3 filter paper in a 5.5 cm in diameter Buchner Funnel under vacuum filtration. The solid residue was left for 24h at room temperature (*ca.* 23°C) to dry and then kept at –18°C until PAL extraction later (Zucker, 1965).

Flowers were assayed for PAL activity following the methods of Cheng and Breen (1991), with the modification that flowers were made into acetone powders according to Zucker (1965). PAL was extracted from acetone powders by gentle stirring at 4°C in 100 mM sodium borate buffer (5 mL g FW^{-1}) containing 5 mM β-mercaptoethanol and 2 mM EDTA. After 1 h of stirring, the solution was filtered through a Whatman No. 1 filter paper and centrifuged at 20,000 x g for 10 min at 4°C.

PAL activity in the final buffer solution was determined by the production of *trans*-cinnamic acid during 1h of incubation at room temperature (Zucker, 1965). Absorbance was measured at 290 nm using a UV-spectrophotometer (ThermoSpectronic Helios γ, Fisher Scientific, Loughborough, UK) (Zucker, 1965). The assay mixture in plastic UV - grade 5 mL cuvettes (Merck Ltd., Poole, UK) consisted of 1 mL of 15 μM of L-phenylalanine (SIGMA, St. Louis, MO, USA), 1.5 mL 30 mM sodium borate buffer

(pH 8.8) and 0.2 - 0.5 mL (depending on enzyme activity) extract solution in a total volume of 3 mL (Cheng and Breen, 1991). L-phenylalanine was added into the mixture after 10 min of pre-incubation at room temperature. A sample without L-phenylalanine was used as the blank (Zucker, 1965). Assays were performed in triplicate. PAL activity was expressed as nmoles *trans*-cinnamic acid formed per gram of fresh weight per hour (nmoles g FW^{-1} h^{-1}).

4.1.2.8 Statistical analysis

Data from experiments A1, A2 and A3 were analysed using a factorial ANOVA model (univariate ANOVA) to compare main factor means. Individual treatment means were compared using the Duncan's multiple range test at $P = 0.05$. The non-parametric Kruskal-Wallis test was used to determine differences in disease severity rating scoring within factors. Data from experiment A4 was analysed using one-way ANOVA. Correlations between senescence and disease variables (i.e. disease severity, lesion numbers and lesion diameters) were carried out using Pearson's correlation test. Statistical analysis was performed in SPSS 9.0 for Windows. Quadratic regressions were used to describe the relationships between chemical treatment concentrations and disease assessment parameters. Quadratic regression analysis and graphic representation in all experiments were performed using Sigmaplot 2000 for Windows.

4.1.3 Results

4.1.3.1 Effect of acibenzolar-S-methyl on artificially inoculated flowers (experiment A1)

Disease severity, lesion number and lesion diameter main factor means varied in response to temperature but to a lesser extend to acibenzolar concentration (Table 4.2).

Table 4.2: Effect of postharvest acibenzolar treatment on *B. cinerea* disease suppression on freesia var. 'Cote d'Azur' flowers (experiment A1). Flowers were treated with 0 (control), 0.15, 0.3 or 0.6 g AI L^{-1} of acibenzolar, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 3 days at 5, 12 and 20°C. Disease assessments were carried out daily over 3 succesive days of incubation. Data for independent treatment means are presented in Figure 4.1.

Factors	**Disease variables**		
	Disease severity (score 0-4) [a]	**Lesion number**	**Lesion diameter (mm)**
1) Temperature (°C) [b]			
5	0.7 a	28 a	0.9 b
12	2.1 c	91 c	0.9 b
20	1.6 b	78 b	0.7 a
2) Acibenzolar concentration (g AI L^{-1})			
0	1.8 b	72 b	1.0 b
0.15	1.4 a	58 a	0.8 a
0.3	1.4 a	72 b	0.7 a
0.6	1.3 a	55 a	0.8 a

[a] Data are main factor means of disease severity, lesion number and lesion diameter.
[b] Within main factor means, numbers followed by the same letter are not significantly different at P = 0.05.

Disease severity and lesion diameter main factor means were significantly ($P < 0.05$) reduced to a small degree at all acibenzolar concentrations compared to untreated controls. However, no significant ($P > 0.05$) differences were detected between acibenzolar concentrations except for lesion numbers being lower at 0.15 and 0.6 versus 0 and 0.3 g AI L^{-1} (Table 4.2). Flowers incubated at 12°C showed significantly ($P < 0.05$) higher disease severity and lesion numbers to those incubated at 5 or 20°C (Table 4.2). Lesion diameters were significantly ($P < 0.05$) higher in flowers incubated at 5 and 12°C compared to those incubated at 20°C (Table 4.2).

Flowers incubated at 12°C showed significantly ($P < 0.05$) higher disease severity scores and lesion numbers to those incubated at 5 or 20°C (Table 4.2). Lesion diameter means were significantly ($P < 0.05$) higher in flowers incubated at 5 and 12°C compared to those incubated at 20°C (Table 4.2). Incubation of freesia flowers at 5°C resulted in no significant ($P > 0.05$) reduction in disease severity and lesion numbers by acibenzolar at all concentrations tested compared to untreated controls (Figure 4.1 A and B; Appendix 4.1.1, Tables A4.1.1.4 and A4.1.1.6). Incubation at 12°C, resulted in significant ($P < 0.05$) lesion diameter reduction in freesia flowers treated with all acibenzolar concentrations compared to untreated controls (Figure 4.1 C; Appendix 4.1.1, Table A4.1.1.8). Incubation of freesia flowers at 20°C gave a significant ($P < 0.05$) reduction of disease severity compared to untreated controls only for 0.6 g AI L^{-1} acibenzolar treatment (Figure 4.1 A, C; Appendix 4.1.1, Tables A4.1.1.4 and A4.1.1.8).

Lesion diameters on detached petals were also measured in experiment A1. Acibenzolar at all 3 concentrations did not provide significant ($P < 0.05$) reduction of lesion diameters on detached petals (Table 4.3). Although significant positive correlations ($P < 0.05$) were recorded between disease severity and senescence and between lesion numbers and senescence, no significant ($P < 0.05$) correlation was found between lesion diameters and senescence (Table 4.4).

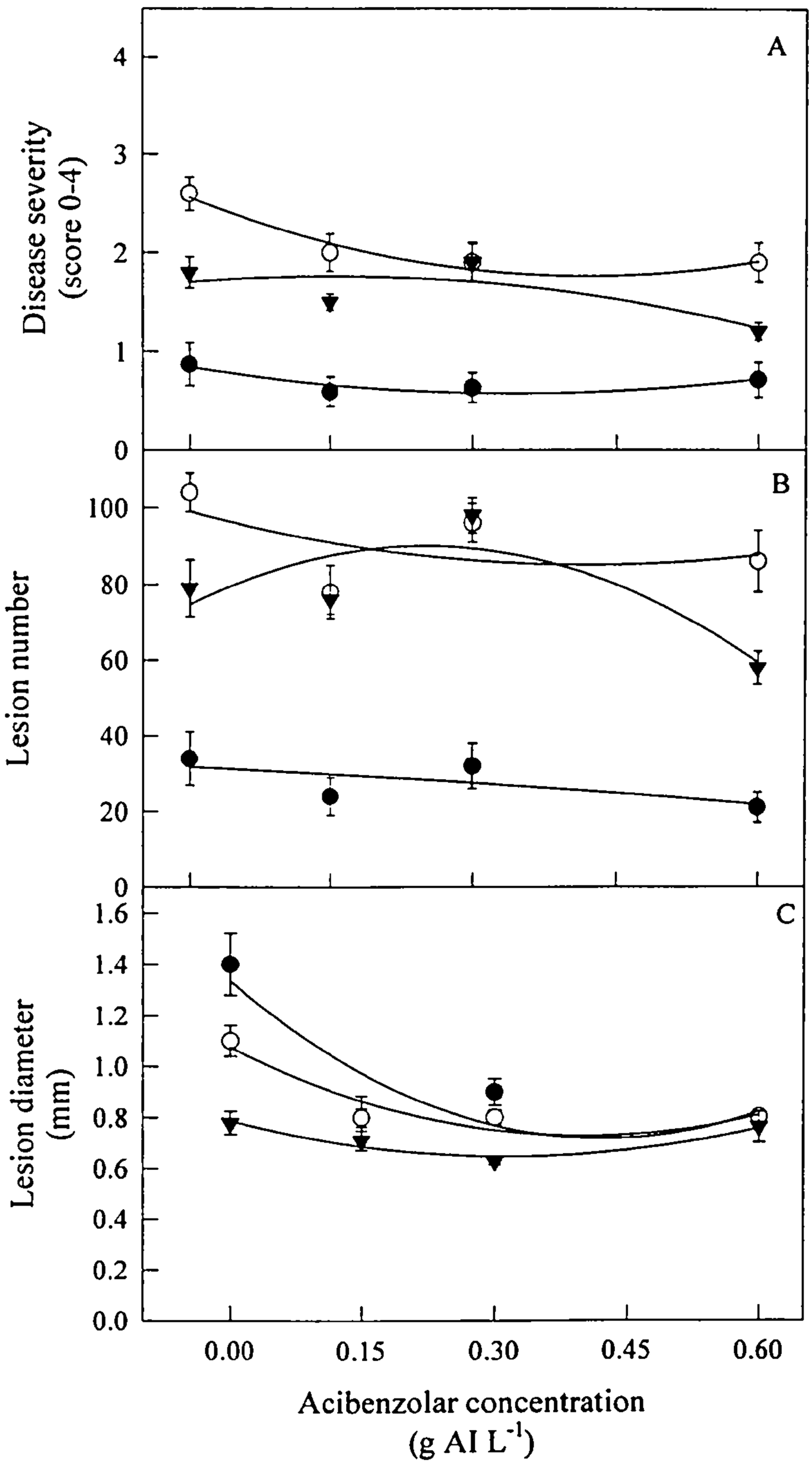

Figure 4.1: Quadratic regressions of disease severity (A), lesion number (B) and lesion diameter (C) of freesia var. 'Cote d'Azur' flowers against treatments with 0 (control), 0.15, 0.3 and 0.6 g AI L^{-1} acibenzolar. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5 (●), 12 (○) and 20°C (▼) (experiment A1). Each data point represents the mean data collected on 3 successive days of incubation. Bars indicate the SE for each treatment (n = 12). Main factor means are presented in Table 4.2 and regression parameters in Appendix 4.1.1, Table A4.1.19.

Table 4.3: Lesion diameter on detached petals of freesia var 'Cote d'Azur' flowers treated with 0 (control), 0.15, 0.3 and 0.6 g AI L^{-1} acibenzolar and incubated at 20°C for 48h (experiment A1).

Acibenzolar concentration (g AI L^{-1})	**Number of flower samples**	**Lesion diameter (mm) [a]**
0	10	4.6 a
0.15	10	4.3 a
0.3	10	4.2 a
0.6	10	5.0 a

[a] Within lesion diameters, numbers followed by the same letter are not significantly different at P = 0.05 (Appendix A4.1.1.10)

Table 4.4: Effect of senescence on disease severity scores, lesion numbers and lesion diameters of freesia var 'Cote d'Azur' flowers treated with 0 (control), 0.15, 0.3 and 0.6 g AI L^{-1} acibenzolar and inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 3 days at 5, 12 and 20°C (experiment A1).

Disease variables	**Number of observations**	**Senescence [a]**
Disease severity	320	0.395 **[b]
Lesion number	320	0.366 **
Lesion diameter	140	0.070 ns

[a] Senescence was measured according to the arbitary scale described in section 4.1.2.5.
[b] Data are results from Pearson's correlation at P = 0.05
** Significance at P = 0.05, ns: not significant at P = 0.05

4.1.3.2 Effect of acibenzolar-S-methyl on non-inoculated (naturally infected) flowers (experiment A2)

Lesion numbers on non-inoculated (naturally infected) freesia var. 'Cote d'Azur' flowers incubated at 5 and 12°C were significantly ($P < 0.05$) reduced after acibenzolar treatment at all concentrations tested (Table 4.5; Appendix 4.1.2, Table A4.1.2.1). However, incubation of acibenzolar treated flowers at 20°C resulted in no significant ($P > 0.05$) lesion number reduction (Table 4.5).

Table 4.5: Effect of postharvest acibenzolar treatment on *B. cinerea* non-inoculated freesia var. 'Cote d'Azur' flowers. Flowers were treated with 0 (control), 0.15, 0.3 and 0.6 g AI L^{-1} acibenzolar and incubated for 3 days at 5, 12 and 20°C (experiment A2). Lesion number assessments were carried out daily. Each data point represents the mean data collected on 3 successive days of incubation.

Temperature (°C)	**Acibenzolar concentration (g AI L^{-1})**	**Lesion number [a]**
5	0	6.6 b
	0.15	1.6 a
	0.3	1.5 a
	0.6	2.0 a
12	0	4.8 b
	0.15	1.1 a
	0.3	2.5 a
	0.6	1.3 a
20	0	2.7 a
	0.15	0.5 a
	0.3	0.9 a
	0.6	1.2 a

[a] For the acibenzolar treatment by temperature interaction, numbers followed by the same letter are not significantly different at $P = 0.0.5$.

4.1.3.3 Effect of acibenzolar-S-methyl on artificially inoculated flowers (experiment A3)

Freesia flowers var. 'Texel' were treated with acibenzolar at 0 (control), 0.15, 0.3, and 0.6 g AI L^{-1}. Disease assessments were carried out on non-inoculated (i.e. naturally infected) or artificially inoculated (10^3, 10^4 or 10^5 *B. cinerea* conidia mL^{-1}) freesia flowers. Treated flowers were incubated at 5, 12 or 20°C. Both acibenzolar and the mixture of acibenzolar + iprodione treatments significantly ($P < 0.05$) reduced disease severity on freesia flowers compared to untreated controls (Table 4.6; Appendix 4.1.3, Tables A4.1.3.1–A4.1.3.4).

Table 4.6: Effect of postharvest acibenzolar treatment on *B. cinerea* disease suppression on freesia var. 'Texel' flowers (experiment A3). Flowers were treated with acibenzolar at 0.15 g AI L^{-1} and iprodione at 0.5 g AI L^{-1} were left non-inoculated (naturally infected) or inoculated with 10^3, 10^4 and 10^5 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C. Disease assessments were carried out daily for 3 days after artificial inoculation. Data for independed treatment means are presented in Figure 4.2.

Factors	**Disease severity (score 0-4)**
1) Inoculum level (*B. cinerea* conidia mL^{-1}) [a]	
0	0.1 a
10^3	0.6 b
10^4	1.9 c
10^5	3.6 d
2) Temperature (°C)	
5	1.3 a
12	1.7 b
20	1.7 b
3) Chemical treatments	
Control (water)	1.8 a
Acibenzolar-S-methyl (0.15 g AI L^{-1})	1.4 c
Iprodione (0.5 g AI L^{-1})	1.6 b
Acibenzolar + Iprodione (0.15 + 0.5 g AI L^{-1})	1.4 c

[a] Within main factor means, numbers followed by the same letter are not significantly different at $P = 0.05$.

Iprodione alone significantly ($P < 0.05$) reduced disease severity compared to control flowers (Table 4.6). In general, flowers treated with acibenzolar alone or with the mixture of acibenzolar + iprodione showed a significant ($P < 0.05$) reduction in disease severity compared to iprodione treated flowers (Table 4.6). Inoculum level had a highly significant ($P < 0.05$) effect on disease severity which increased proportionally with inoculum increases (Table 4.6).

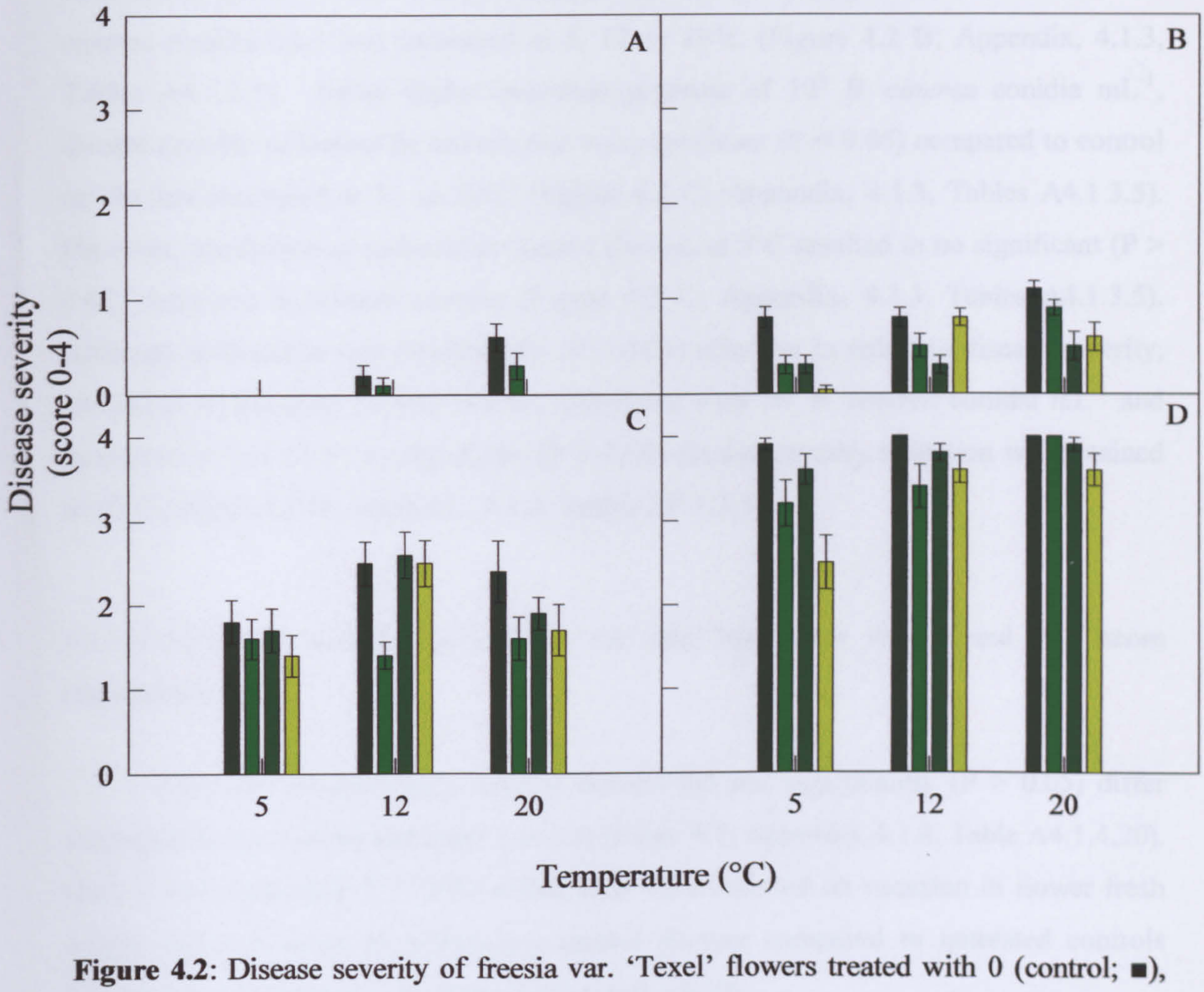

Figure 4.2: Disease severity of freesia var. 'Texel' flowers treated with 0 (control; ■), acibenzolar-S-methyl at 0.15 g AI L^{-1} (■), iprodione at 0.5 g AI L^{-1} (■) and acibenzolar + iprodione at 0.15 + 0.5 g AI L^{-1} (■) and incubated at 5, 12 and 20°C. Flowers were left non-inoculated (A) or inoculated with 10^3 (B), 10^4 (C) and 10^5 (D) *B. cinerea* conidia mL^{-1}. Bars indicate SE of each treatment (n = 16). Main factor means are presented in Table 4.6.

Disease severity was reduced by acibenzolar only under specific conditions (Figure 4.2 A, B, C, D; Appendix, 4.1.3, Tables A4.1.3.5). Acibenzolar alone and the mixture of acibenzolar + iprodione did not significantly ($P > 0.05$) reduce disease severity compared to control, on non-inoculated (naturally infected) flowers incubated at 5, 12 or 20°C (Figure 4.2 A; Appendix, 4.1.3, Tables A4.1.3.5). Moreover, acibenzolar and acibenzolar + iprodione treatments did not significantly ($P > 0.05$) reduce disease severity, compared to the untreated control flowers artificially inoculated with 10^3 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 or 20°C (Figure 4.2 B; Appendix, 4.1.3, Tables A4.1.3.5). Under higher inoculum pressure of 10^4 *B. cinerea* conidia mL^{-1}, disease severity reduction by acibenzolar was significant ($P < 0.05$) compared to control on flowers incubated at 12 or 20°C (Figure 4.2 C; Appendix, 4.1.3, Tables A4.1.3.5). However, incubation of acibenzolar treated flowers at 5°C resulted in no significant ($P > 0.05$) reduction in disease severity (Figure 4.2 C; Appendix, 4.1.3, Tables A4.1.3.5). Although acibenzolar was significantly ($P < 0.05$) effective in reducing disease severity, compared to untreated control flowers inoculated with 10^5 *B. cinerea* conidia mL^{-1} and incubated at 5 or 12°C, no significant ($P > 0.05$) disease severity reduction was obtained at 20°C (Figure 4.2 D; Appendix, 4.1.3, Tables A4.1.3.5).

4.1.3.4 Effect of acibenzolar-S-methyl on vase life, fresh weight and wilt score (experiment A4)

Vase life of acibenzolar treated flowers did not significantly ($P > 0.05$) differ compared to these of the untreated controls (Table 4.7; Appendix 4.1.4, Table A4.1.4.20). Only a few significant ($P < 0.05$) differences were detected on occasion in flower fresh weight and wilt score of acibenzolar treated flowers compared to untreated controls (Figure 4.3; Appendix 4.1.4, Tables A4.1.4.1–4.1.4.19).

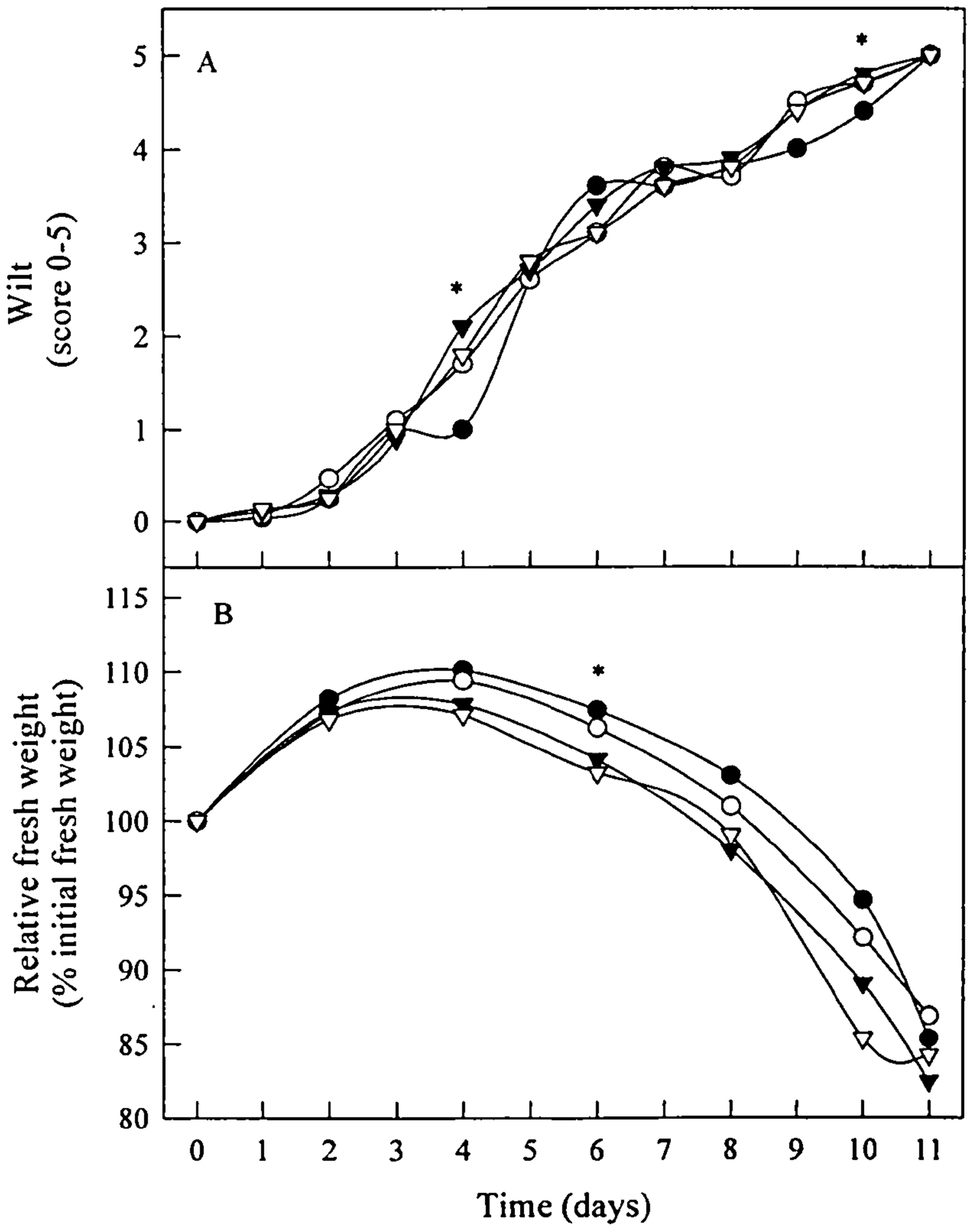

Figure 4.3: Wilt score (A) and relative fresh weight (B) of freesia flowers treated with 0 (●), 0.15 (○), 0.3 (▼) and 0.6 (▽) g AI L^{-1} acibenzolar and incubated at 20°C (experiment A4). Stars indicate significant difference between treatments at P = 0.05 (Appendix 4.1.4, Tables A4.1.4.1–A4.1.4.19).

Table 4.7: Vase lives of freesia var. 'Cote d'Azur' flowers treated with 0 (control), 0.15, 0.3 and 0.6 g AI L^{-1} acibenzolar and incubated at 20°C (experiment A4).

Acibenzolar concentration (g AI L^{-1})	**Vase life [a] (days)**
0	10.6 a
0.15	10.4 a
0.3	10.3 a
0.6	10.1 a

[a] Within vase life variable, numbers followed by the same letter are not significantly different at P = 0.05.

4.1.3.5 Effect of acibenzolar-S-methyl on PAL activity (experiment A5)

PAL activity of acibenzolar treated flowers was fairly constant throughout incubation. Moreover, it was not significantly ($P > 0.05$) higher than that of the untreated control flowers (Figure 4.4; Appendix 4.1.5, Tables A4.5.1.1–4.5.1.4). On day 3 of incubation, PAL activities of both acibenzolar treated and untreated control flowers showed the highest levels of *trans*-cinnamic acid g FW^{-1} at 1003 and 725 nmoles, respectively.

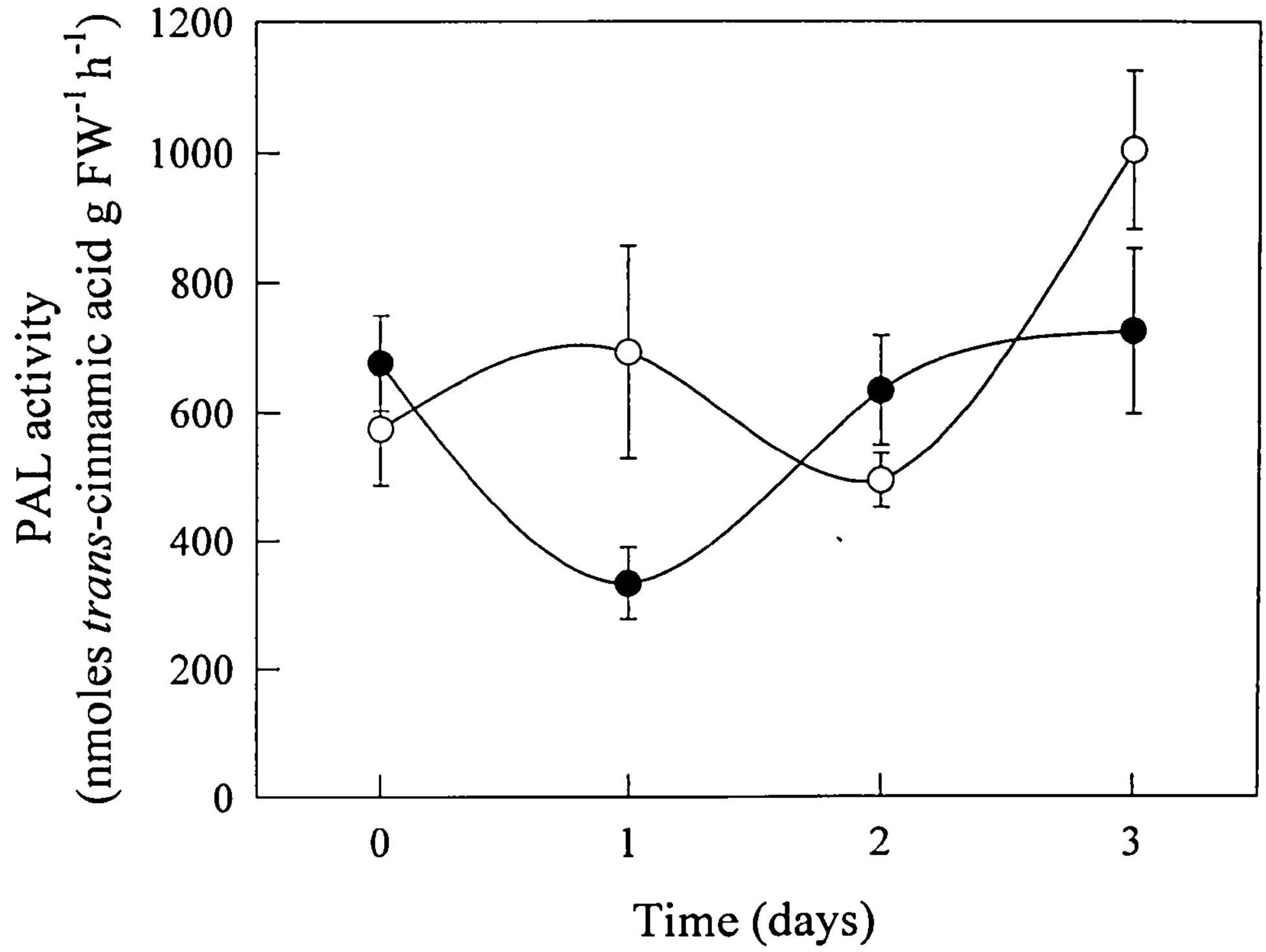

Figure 4.4: Phenylalanine ammonia lyase (PAL) activity over time of freesia var. 'Texel' treated with 0.15 g AI L^{-1} (○) acibenzolar or left untreated (●) and incubated for 3 days at 20°C (Experiment 5). Bars indicate the SE (n = 3).

4.1.3.6 Effect of acibenzolar-S-methyl on *B. cinerea* mycelium growth, conidial germination and germ tube elongation (experiment A6)

There was a significant ($P < 0.05$) inhibition of *B. cinerea* colony diameter growth *in-vitro* in acibenzolar-supplemented media (Figure 4.5; Appendix 4.1.6, Table A4.1.6.3). Moreover, acibenzolar significantly ($P < 0.05$) inhibited *B. cinerea* germ tube elongation, irrespective of the concentration used. (Table 4.8; Appendix 4.1.6, Table A4.1.6.2). However, no inhibitory effects of acibenzolar were observed on *B. cinerea* conidial germination except at 0.6 g AI L^{-1} (Table 4.8; Appendix 4.1.6, Table A4.1.6.1).

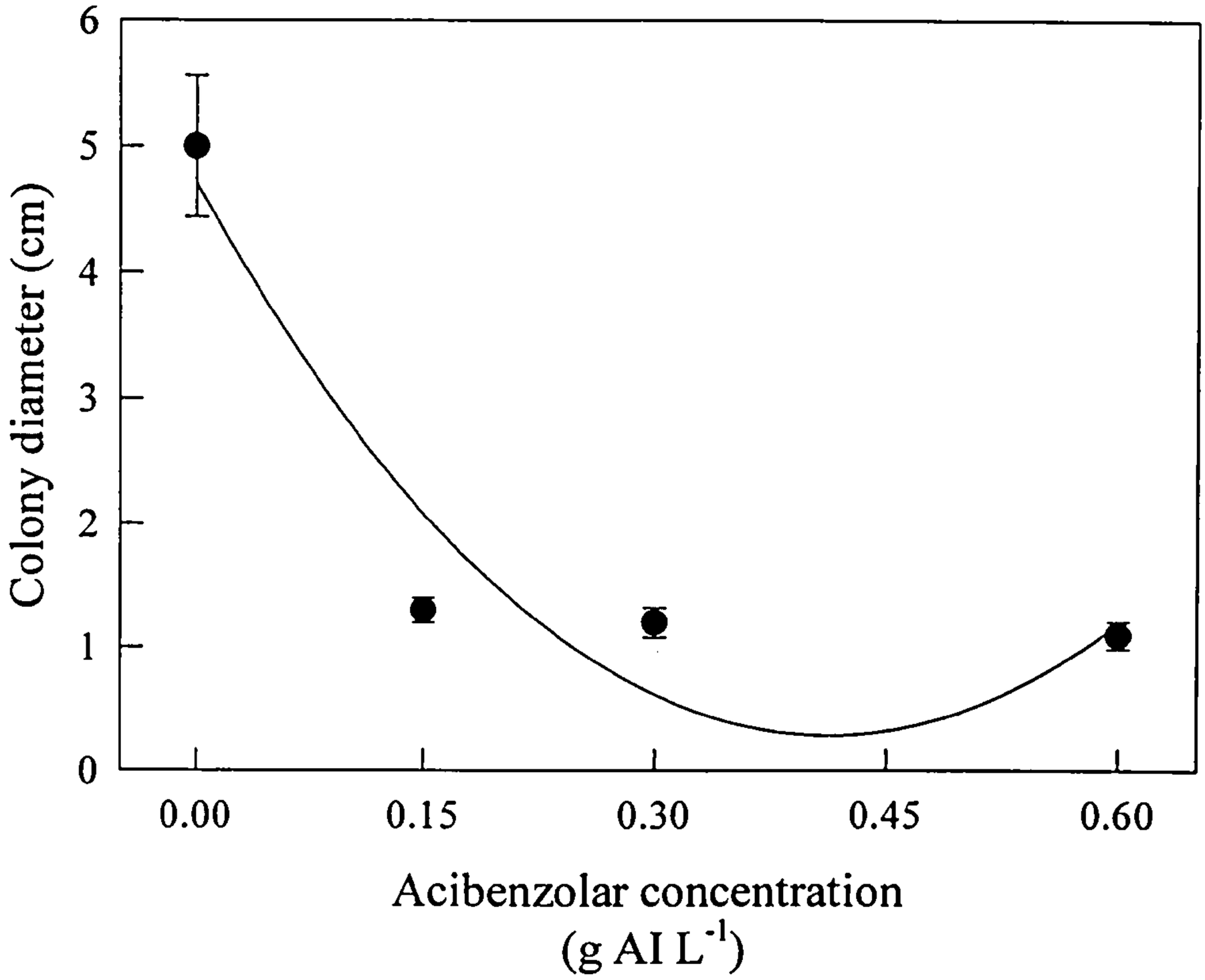

Figure 4.5: *In-vitro* mycelium growth of *B. cinerea* isolate (BcF1) on ½ PDA supplemented with acibenzolar at 0 (control), 0.15, 0.3 and 0.6 g AI L^{-1} (experiment A6). Data are means of 5 days of incubation at 20°C in the dark. Regression line: $y = 4.7 - 21.43x + 25.96x^2$, $R^2 = 0.90$. Bars indicate the SE (n = 25).

Table 4.8: Proportion (%) of *B. cinerea* conidial germination and germ tube elongation *in vitro* at 4 acibenzolar concentrations (experiment A6). Conidial suspensions were incubated for 12h at 20°C in the dark.

Acibenzolar concentration (g AI L^{-1})	***B. cinerea* conidial germination (%) [a]**	**Germ tube elongation (µm)**
0	26 ab	46.1 a
0.15	22 a	27.8 b
0.3	29 ab	25.0 b
0.6	34 b	19.5 b

[a] Within each variable, numbers followed by the same letter are not significantly different at P = 0.05.

4.1.4 Discussion

Postharvest treatments with acibenzolar provided only very limited protection of freesia flowers against *B. cinerea*. Acibenzolar reduced disease severity, lesion numbers and lesion diameters in only a few cases. Acibenzolar was seemingly effective compared to the untreated control when applied at 0.15 g AI L^{-1}. There is no analogous published research on crops treated postharvest with acibenzolar. However, the efficacy of preharvest foliar sprays with acibenzolar on postharvest diseases in rock melons, strawberries and passionfruit has been reported by Huang *et al.* (2000), Terry and Joyce (2000) and Willingham *et al.* (2002), respectively. Pre-harvest acibenzolar applications gave suppression of postharvest infection by *Fusarium* spp., *Alternaria* spp., *Rhizopus* spp., *Trichothecium* sp. on rock melons and on *B. cinerea* on strawberries. Postharvest acibenzolar application might not be as effective in detached organs due to changes in their physiology and/or may be too late in plant organ development to induce systemic responses that could potentially lead to *B. cinerea* disease suppression. Similar limited effect of acibenzolar postharvest treatment on strawberries has been reported by Terry (2002). Likewise, Bokshi *et al.* (2000) found that although pre-harvest acibenzolar application significantly ($P < 0.05$) reduced Alternaria leaf spot and powdery mildew of potatoes, treatment of harvested tubers with acibenzolar did not result in postharvest disease suppression (Bokshi *et al.*, 2000). Moreover, postharvest application of acibenzolar on harvested plant material may not accomodate the criterion that plant activators require a lag time between application and systemic protection described by Kessmann *et al.* (1996) and Ruess *et al.* (1996). Nontheless, in contrast to artificially inoculated flowers, natural infection was significantly ($P < 0.05$) reduced after acibenzolar treatment and incubation at 5 and 12°C. This observation does not accord with an observation that postharvest treatment of strawberry fruits with acibenzolar failed to reduce natural infection by *B. cinerea* stored at 5°C (Terry, 2002). Jarvis (1980b) reported that biochemical processes, such as hypersensitive response and accumulation of phytoalexins, can be either incomplete or proceed only slowly in plant tissue under low storage temperature conditions.

The results of the present study show *in-vitro* sensitivity of *B. cinerea* isolate BcF1 to acibenzolar. Likewise, Terry and Joyce (2000) showed that acibenzolar had direct antimicrobial effects on *B. cinerea* mycelial growth. However, according to Friedrich *et al.* (1996) mycelial radial growth of *B. cinerea* was not inhibited when grown on acibenzolar agar supplemented with at 0.294 g AI L^{-1}. In the present study, it is more likely that limited *B. cinerea* disease suppression on freesia flowers at storage temperatures may be due to a direct acibenzolar effect rather than to SAR induction. As PAL activity was generally similar in both control and acibenzolar treated flowers it is unclear whether postharvest acibenzolar treatment induced SAR or other responses.

Overall iprodione was inconsistent over incubation temperatures and inoculum levels. Iprodione was generally effective in suppressing *B. cinerea* disease severity on freesia flowers inoculated with 10^3 and incubated at 5, 12 and 20°C and when inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C as compared to untreated controls. These results agree with earlier findings on the postharvest efficacy of the fungicide iprodione in suppressing grey mould disease caused by *B. cinerea* on rose and Geraldton waxflower (Elad, 1988; Elad *et al.*, 1993a; Taylor *et al.*, 1999). However, in most cases acibenzolar at 0.15 g AI L^{-1} provided similar or even better (flowers inoculated with 10^4 and incubated at 12°C) *B. cinerea* disease suppression to that provided after iprodione treatment.

4.2 SUPPRESSION BY POSTHARVEST TREATMENT WITH METHYL JASMONATE

4.2.1 Introduction

Methyl jasmonate (MeJA) is another plant activator recently used in both pre- and postharvest experiments. MeJA has been tested as a postharvest application on different horticultural products including cut roses (Table 2.6). MeJA applied as a postharvest pulse to six rose cultivars (e.g. Mercedes, Europa, Lambada, Frisco, Sacha and Eskimo) at a range of concentrations between 100 and 600 μM gave a systemic protection against

B. cinerea (Meir *et al.*, 1998). MeJA significantly reduced lesion size caused by *B. cinerea*. JA and MeJA were also tested as postharvest dips against *Penicillium digitatum* (Pers.:Fr.) Sacc. on grapefruit (Droby *et al.*, 1999). MeJA did not show any direct antifungal activity *in-vitro* suggesting that disease suppression occurred through natural resistance induction (Droby *et al.*, 1999).

The effects of MeJA against *B. cinerea* on freesia flowers via its potential to induce defence responses were investigated. MeJA was tested under a range of temperatures, inoculum levels and also *in-vitro*. Gaseous, pulse and spray application methods were tested. Disease severity and vase life were evaluated

4.2.2 Materials and methods

4.2.2.1 Plant material

Freesia flowers of var 'Cote d' Azur' were provided by Zwetsloots & Sons Ltd. (UK) (Appendix 2.2, Plate A2.1). Flowers were at the commercial harvest stage with all buds still closed (Appendix 2.2, Plate A2.2). They were processed in the laboratory approximately 24h after harvest.

4.2.2.2 Artificial inoculation

In all experiments the cut freesia flowers were either artificially inoculated or left non-inoculated (i.e. naturally infected flowers). Conidial suspensions of *B. cinerea* were prepared as outlined in section 3.2.2.2. Suspensions were adjusted to 10^4 *B. cinerea* conidia mL^{-1} with a haematocytometer and checking two counts per conidial suspension.

4.2.2.3 Experiment design

In experiments M1-9, the effects of MeJA application mode (gaseous, pulse, spray) on disease caused by *B. cinerea* were studied with 5 replicate flowers per treatment (Table 4.9).

Table 4.9: Experiment number, freesia variety, number of replications, inoculum treatment concentration, assessment parameters and other treatment variables pertaining in the MeJA experiments.

Experiment	**Replications**	**Inoculum concentration (No. of conidia mL^{-1})**	**Chemical application mode**	**Assessments**	**Factors** [b]
M1	5	10^4	Gas	Disease [a]	T x M
M2	5	- [c]	Gas	Disease	T x M
M3	5	-	Gas	Vase life	M
M4	5	10^4	Pulse	Disease	T x M
M5	5	-	Pulse	Disease	T x M
M6	5	-	Pulse	Vase life	M
M7	5	10^4	Spray	Disease	T x M
M8	5	-	Spray	Disease	T x M
M9	5	-	Spray	Vase life	M
M10	5	2×10^6	Solution	*In-vitro*	M

[a] Disease assessments include disease severity, lesion number and lesion diameter.
[b] T: Temperature, M: Methyl jasmonate treatment.

The experiments were arranged in a CRB design with 5 blocks and 4 sample flowers in each block. Experiments M1, M2, M4, M5, M7 and M8, were two-factor designs with temperature (5, 12 and 20°C) and MeJA treatment (gas: 0, 0.025, 0.05 and 0.1 μL MeJA L^{-1}, pulse: 0, 200, 400 and 600 μM, spray: 0, 200, 400 and 600 μM) as factors. Experiments M3, M6, and M9, were arrange inside a vase life room in a CR

design with MeJA treatment (gas: 0, 0.025, 0.05 and 0.1 μL MeJA L^{-1}, pulse: 0, 200, 400 and 600 μM, spray: 0, 200, 400 and 600 μM) as factor.

4.2.2.4 Chemical treatments

MeJA (95%, Aldrich, Milwaukee, USA) was applied to freesia var. 'Cote d'Azur' flowers in gas, pulse or spray forms.

Gas treatment

In experiments M1, M2, and M3, MeJA gassing was performed in gas-tight PVC containers (60 x 60 x 90 cm). Gaseous MeJA was applied by pipetting an appropriate aliquot of 1% (v/v) MeJA liquid in ethanol onto a cotton plug inside the container and then allowing the MeJA to evaporate (Thomma *et al.*, 2000). Gaseous MeJA concentrations were calculated based on the assumption that MeJA evaporated completely. The treatments were at the rates of 0 (control), 0.025, 0.05 and 0.1 μL MeJA L^{-1} (Thomma *et al.*, 2000). Freesia flowers were stood for 24h at 20°C, in vases containing tap water, within the gas-tight containers (Thomma *et al.*, 2000). After 24h, flowers were removed from the containers and either inoculated with *B. cinerea* conidial suspension (10^4 conidia mL^{-1}; experiment M1) or left non-inoculated (i.e. naturally infected) (experiment M2). They were covered with transparent plastic bags to maintain *ca.* 100% RH (section 4.1.2.3). Thereafter, artificially inoculated and non-inoculated flowers were incubated at 5, 12 or 20°C in the dark. In experiment M3 the effects of gaseous MeJA on the vase life was studied on non-inoculated freesia flowers incubated at 20°C and 60 ± 10% RH under a diurnal light (12h) photoperiod.

Pulse treatment

In experiments M4, M5, and M6, 0 (control), 200, 400 and 600 μM MeJA solutions were prepared according to the method described by Meir *et al.* (1998). Briefly, MeJA was dissolved in 0.2% of ethanol in distilled water. Same amount of ethanol was added to the control solutions (i.e. 0 μM MeJA). Freesia flowers were divided into four bunches and stood into 1.5 L glass jars containing 500 mL MeJA solution. The lid of each jar was sealed with plastic transparent film to minimise evaporation of the MeJA solution and jars were held at 20°C (Meir *et al.*, 1998). After 24h, flowers were either inoculated with a *B. cinerea* conidial suspension (10^4 conidia mL^{-1}; experiment M4) or left non-inoculated (i.e. naturally infected) (experiment M5) and covered with transparent plastic bags to maintain *ca.* 100% RH (section 4.1.2.3). Subsequently, the artificially inoculated and non-inoculated flowers were incubated at 5, 12 or 20°C in the dark. In experiment M6 examining the effects of pulse of MeJA on vase life, non-inoculated pulse treated flowers were incubated at 20°C and 60 ± 10% RH under a diurnal (12h) photoperiod.

Spray treatment

In experiments M7, M8, and M9, 0 (control), 200, 400 and 600 μM MeJA solutions were prepared according to Meir *et al.* (1998). MeJA was dissolved in 0.2% ethanol in distilled water and the same amount of ethanol was added to the control solutions for 0 μM MeJA. Freesia flower bunches were sprayed with a 2.5 L hand-pump until incipient run-off and then stood into 284 mL plastic vases containing tap water for 24h at 20°C (Thomma *et al.*, 2000). After 24h, the flowers were either inoculated with a *B. cinerea* suspension (10^4 conidia mL^{-1}; experiment M7) or left non-inoculated (experiment M8). Then they were covered with transparent plastic bags to maintain *ca.* 100% RH (section 4.1.2.3). Then both the artificially inoculated or non-inoculated (i.e. naturally infected) flowers were incubated at 5, 12 or 20°C in the dark. In experiment M9 examining the effects of MeJA applied as spray on the vase life, the non-inoculated flowers were incubated at 20°C and 60 ± 10% RH under a diurnal light (12h) photoperiod.

4.2.2.5 Assessments

Disease

Disease severity on artificially inoculated freesia flowers was evaluated daily using the arbitrary scale described in section 3.2.2.4. The diameters of 10 randomly selected lesions per flower were measured each day following artificial inoculation by the method described in section 3.2.2.4. In experiments M2, M5 and M8 only the number of lesions was recorded on non-inoculated naturally infected flowers.

Flower development and senescence

Flower development and senescence was scored every day after inoculation using the scale described in section 3.2.2.4.

Vase life

In experiments M3, M6 and M9, vase life (days), flower fresh weight and wilt score were recorded. Flower fresh weight was recorded every second day as described in section 4.1.2.5. These data are presented as proportion (%) change from the initial fresh weight. Wilt score was recorded daily as described in section 4.1.2.5.

Detached petal bioassays

Lesion diameter on detached freesia petals was recorded only in experiments M1, M4 and M7 according to Meir *et al.* (1998) as described in section 4.1.2.5.

4.2.2.6 *In-vitro* MeJA activity

B. cinerea *mycelial growth*

The effects of sterile MeJA solutions on *B. cinerea* mycelial growth were tested. MeJA solutions of 0 (control), 200, 400 and 600 μM were prepared and measured as described in section 4.2.1.6.

B. cinerea *conidial germination and germ tube elongation*

The direct antifungal effect of MeJA on *B. cinerea* conidial germination and germ tube elongation was tested. MeJA solutions were prepared at the concentrations of 0 (control), 200, 400 and 600 μM and conidial germination, germ tube elongation was measured as described in section 4.2.1.6.

4.2.2.7 Statistical analysis

Data from experiments M1-M9 were analysed as described in section 4.1.2.8 using SPSS 9.0 for Windows. Quadratic regression analysis and graphic representation in all experiments were performed using Sigmaplot 2000 for Windows. Data are presented either as main factor means in tables and corresponding individual treatment means in figures or as individual treatment means in figures or tables.

4.2.3 Results

4.2.3.1 Effects of gaseous MeJA on artificially inoculated flowers (experiment M1)

Disease severity, lesion numbers and lesion diameters were temperature dependant (Table 4.10). Based on main factor means, the proportion of disease suppression by MeJA was increased with storage temperature.

Table 4.10: Effect of postharvest methyl jasmonate (MeJA) treatment on *B. cinerea* disease suppression on freesia var. 'Cote d'Azur' flowers (experiment M1). Flowers were gassed with 0 (control), 0.025, 0.05 or 0.1 μL L^{-1} MeJA, inoculated with *B. cinerea* at 10^4 conidia mL^{-1} and incubated for 3 days at 5, 12 and 20°C. Disease assessments were carried out daily for 3 successive days after artificial inoculation. Data for independent treatment means are presented in Figure 4.6.

Factors	**Disease variables**		
	Disease severity (score 0-4) [a]	**Lesion number**	**Lesion diameter (mm)**
1) Temperature (°C) [b]			
5	0.4 a	11 a	0.67 a
12	0.9 c	31 c	0.80 b
20	0.6 b	24 b	0.71 a
2) Gaseous MeJA concentration (μL L^{-1})			
0	0.9 b	30 b	0.94 c
0.025	0.6 a	20 a	0.74 b
0.05	0.5 a	20 a	0.65 a
0.1	0.4 a	17 a	0.59 a

[a] Data are main factor means of disease severity, lesion number and lesion diameter.
[b] Within main factor means, numbers followed by the same letter are not significantly different at P = 0.05.

Although disease severity, lesion numbers and lesion diameters decreased with increasing MeJA concentration there were no significant ($P > 0.05$) differences between MeJA concentration means for these disease assessment parameters (Table 4.10).

Incubation of freesia flowers at 12°C resulted in significantly ($P > 0.05$) higher disease severity, lesion numbers and lesion diameters compared to flowers incubated at 5 or 20°C (Table 4.10). Disease severity, lesion numbers and lesion diameters of flowers treated with 0.1 μL MeJA L^{-1} were reduced by 56, 43 and 37%, respectively compared to untreated controls (Table 4.10). Gaseous MeJA was more effective in reducing disease severity, lesion numbers and lesion diameters at 20°C than at 12°C (Figure 4.6 A, B and C; Appendix 4.2.1, Tables A4.2.1.4, A4.2.1.6 and A4.2.1.8) (Plate 4.1). At 5°C, MeJA was ineffective in reducing disease severity, lesion numbers or lesion diameters (Figure 4.6 A, B and C; Appendix 4.2.1, Tables A4.2.1.4, A4.2.1.6 and A4.2.1.8).

Plate 4.1: Lesions of *B. cinerea* on artificially inoculated freesia flowers treated with 0.1 μL L^{-1} gaseous MeJA (A) or left un-gassed (control) (B) and incubated for 2 days at 20°C.

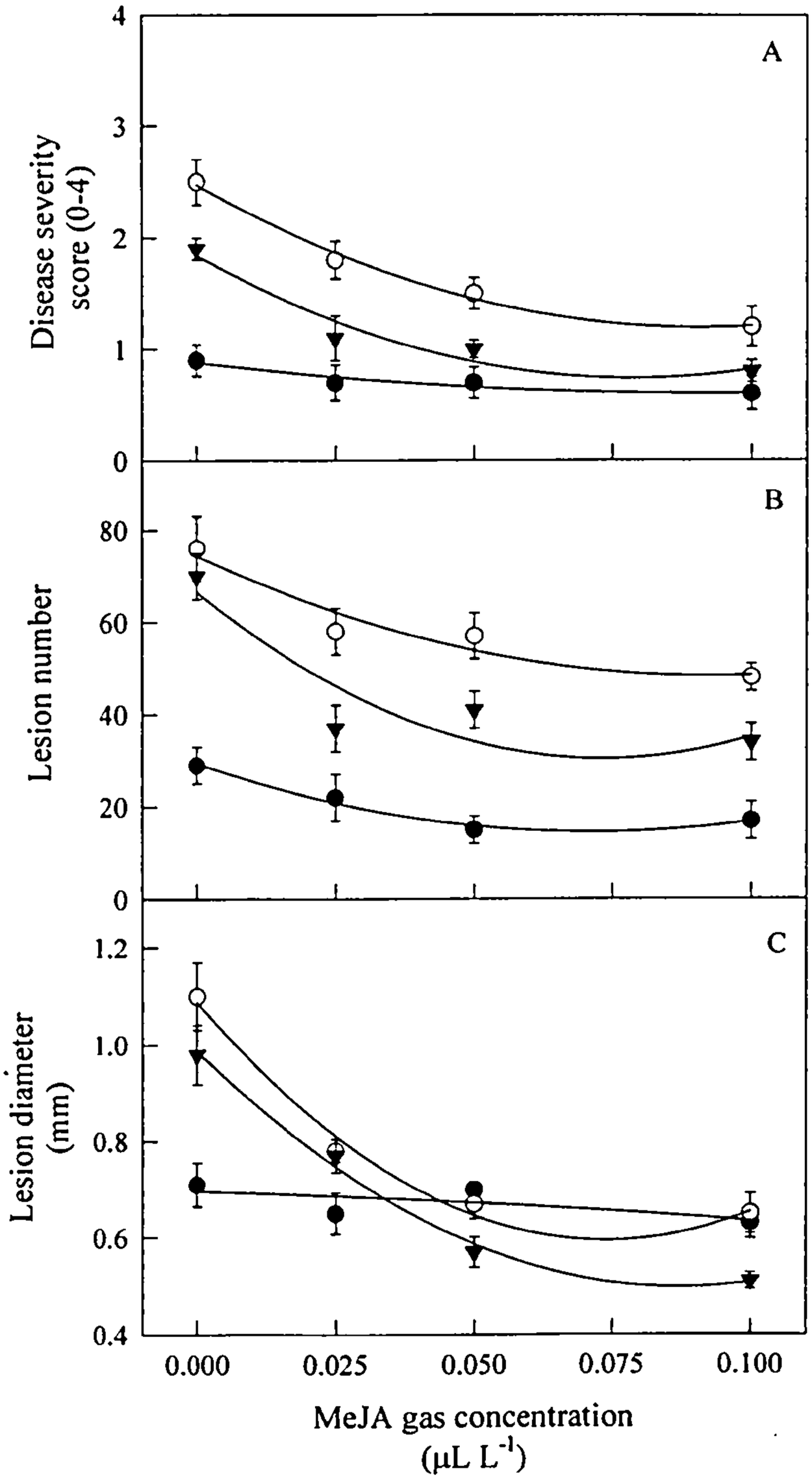

Figure 4.6: Quadratic regression of disease severity (A), lesion number (B) and lesion diameter (C) of freesia var. 'Cote d'Azur' flowers gassed with 0 (control), 0.025, 0.05 and 0.1 µL L^{-1} MeJA, inoculated with 10^4 *B. cinerea* conidia $mL^{-1}$and incubated at 5 (●), 12 (○) and 20°C (▼) (experiment M1). Each data point represents the mean data collected on 3 successive days of incubation. Bars indicate the SE for each treatment (n = 12). Main factor means are presented in Table 4.10 and regression parameters in Appendix 4.2.1, Table A4.2.1.9.

Lesion diameters on detached petals were significantly ($P < 0.05$) reduced on flowers treated with MeJA irrespective of the concentration used (Table 4.11; Appendix 4.2.1, Table A4.2.1.10). Greatest lesion diameter reduction was observed on flowers treated with 0.1 μL MeJA L^{-1} (Table 4.11). However, there was no significant ($P > 0.05$) difference between MeJA concentrations in terms of lesions diameters on detached petals (Table 4.11). Although significant ($P < 0.01$) positive correlations were found between disease severity and senescence and between number of lesions and senescence, no significant ($P < 0.01$) correlation was recorded between lesion diameters and flower senescence (Table 4.12).

Table 4.11: Lesion diameter on detached petals of freesia var 'Cote d'Azur' flowers gassed with 0 (control), 0.025, 0.05 and 0.1 μL L^{-1} MeJA and incubated at 20°C for 48h (experiment M1).

Gaseous MeJA concentration (μL L^{-1})	**Number of flower samples**	**Lesion diameter (mm) [a]**
0	10	5.1 b
0.025	10	4.0 a
0.05	10	3.7 a
0.1	10	3.2 a

[a] Within lesion diameters, numbers followed by the same letter are not significantly different at P = 0.05.

Table 4.12: Effect of senescence on disease severity, lesion numbers and lesion diameters of freesia var 'Cote d'Azur' flowers gassed with MeJA, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 3 days at 5, 12 and 20°C (experiment M1).

Disease variables	**Number of observations**	**Senescence [a]**
Disease severity	288	0.215 **[b]
Lesion number	288	0.277 **
Lesion diameter	116	0.905 ns

[a] Senescence was measured according to the arbitary scale described in section 4.1.2.5.
[b] Data are results from Pearson's correlation at P = 0.05
** Significance at P = 0.05, ns: not significant at P = 0.05

4.2.3.2 Effects of gaseous MeJA on non-inoculated naturally infected flowers (experiment M2)

MeJA, irrespective of the concentration tested, effectively protected non-inoculated flowers incubated at 5°C (Table 4.13; Appendix 4.2.2, Table A4.2.2.1). However, in contrast, none of the three MeJA concentrations used significantly ($P > 0.05$) reduced lesion numbers on non-inoculated flowers incubated at 12 or 20°C (Table 4.13).

Table 4.13: Effect of postharvest MeJA gaseous treatment on non-inoculated (naturally infected) freesia var. 'Cote d'Azur' flowers (experiment M2). Flowers were gassed with 0 (control), 0.025, 0.05 or 0.1 μL L^{-1} MeJA and incubated at 5, 12 and 20°C. Lesion number assessments were carried out daily. Each data point represents the mean data collected on 3 successive days of incubation.

Temperature (°C)	Gaseous MeJA concentration (μL L^{-1})	Lesion number [a]
5	0	3.8 d
	0.025	1.3 abc
	0.05	1.1 ab
	0.1	1.3 abc
12	0	2.4 bc
	0.025	2.4 bc
	0.05	1.8 abc
	0.1	1.2 abc
20	0	1.1 ab
	0.025	0.4 a
	0.05	2.7 cd
	0.1	1.8 abc

[a] For the MeJA by temperature interaction, numbers followed by the same letter are not significantly different at $P = 0.05$.

4.2.3.3 Effects of gaseous MeJA on freesia vase life (experiment M3)

Wilt scores on days 2, 3, 4, 5 and 6 for MeJA treated flowers were also significantly ($P < 0.05$) lower to that of the untreated control flowers (Figure 4.7A; Appendix A4.2.3, Tables A4.2.3.1–A4.2.3.10).

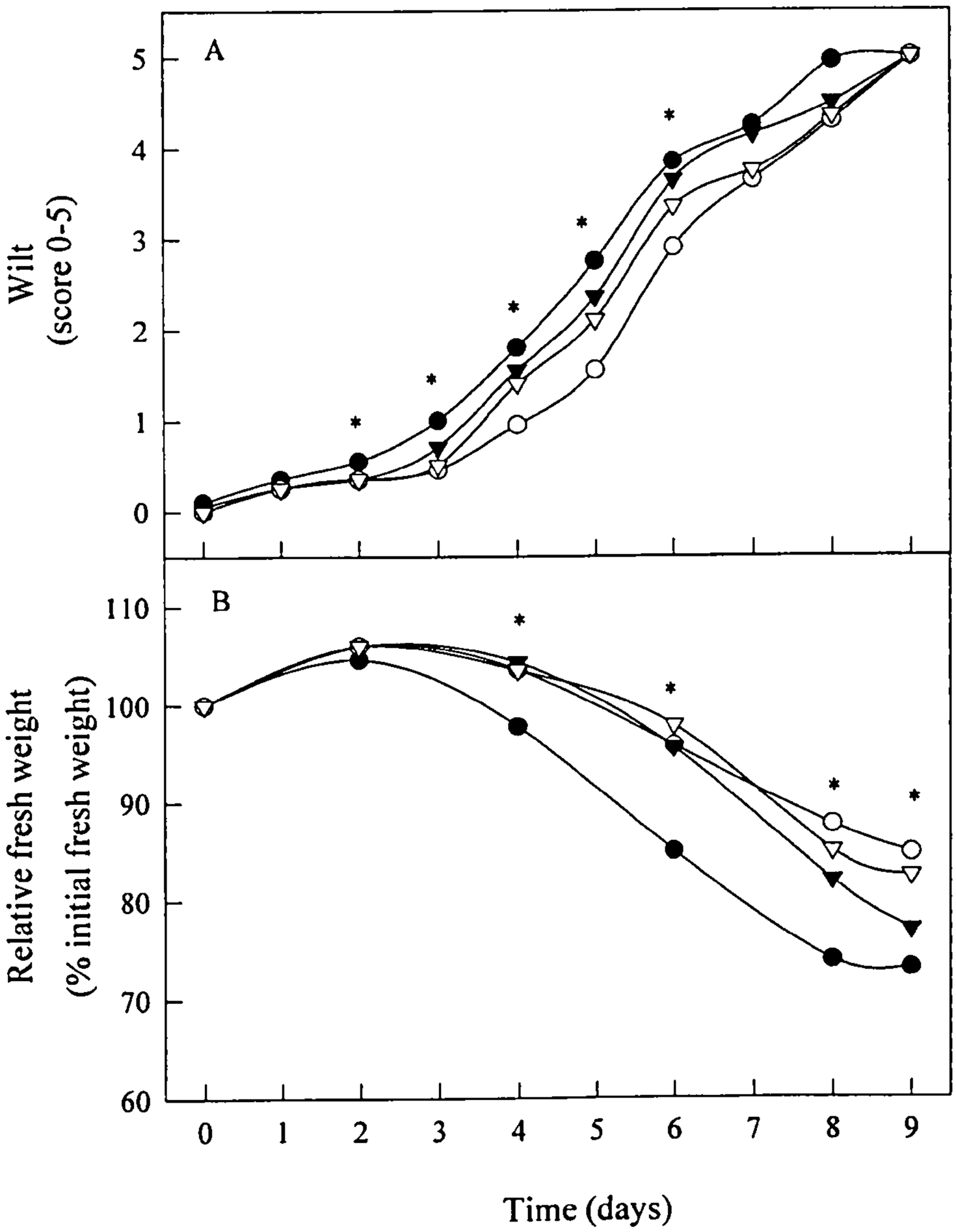

Figure 4.7: Wilt score (A) and relative fresh weight (B) of freesia flowers gassed with 0 (●), 0.025 (○), 0.05 (▼) and 0.1 (▽) μL L^{-1} MeJA and incubated at 20°C (experiment M3). Stars indicate significant difference between treatments at $P = 0.05$ (Appendix 4.2.4, Table A4.2.3.1-A4.2.3.16).

Relative fresh weight on days 4, 6, 8, and 9 of vase life for MeJA treated flowers was also significantly ($P < 0.05$) higher to that of the untreated control (Figure 4.7B; Appendix A4.2.3, Tables A4.2.3.11–A4.2.3.16). Vase lives of flowers treated with MeJA at 0.025 or 0.1 μL L^{-1} were increased significantly ($P < 0.05$) increased by 1 day compared with the untreated controls (Table 4.14; Appendix A4.2.3, Table A4.2.3.17). No phytotoxicity symptoms appeared after MeJA treatment at any of concentrations used.

Table 4.14: Vase lives (days) of freesia var. 'Cote d'Azur' flowers gassed with 0 (control), 0.025, 0.05 and 0.1 μL L^{-1} MeJA and incubated at 20°C (experiment M3).

MeJA concentration (μL L^{-1} air)	**Vase life [a] (days)**
0	10.2 a
0.025	11.2 b
0.05	10.8 ab
0.1	11.2 b

[a] Within vase life variable, numbers followed by the same letter are not significantly different at $P = 0.05$.

4.2.3.4 Effect of pulse MeJA treatment on artificially inoculated flowers (experiment M4)

MeJA pulsed for 24h at 20°C and 200 and 400 μM, significantly ($P < 0.05$) reduced disease severity, lesion numbers and lesion diameters on freesia flowers (Table 4.15). Although MeJA treatment at 600 μM did not affect disease severity or lesion numbers it significantly ($P < 0.05$) reduced lesion diameters (Table 4.15). Temperature significantly affected *B. cinerea* disease. Freesia flowers incubated at 5°C, showed significantly ($P < 0.05$) reduced disease severity, lesion number and lesion diameter factor means (Table 4.15). Incubation of flowers at 12°C resulted in disease severity and lesion numbers similar to those of flowers incubated at 20°C (Table 4.15). Lesion diameters on flowers incubated at 12°C were significantly ($P < 0.05$) larger compared to those on flowers incubated at 20°C (Table 4.15).

Table 4.15: Effect of postharvest methyl jasmonate (MeJA) treatment on *B. cinerea* disease suppression on freesia var. 'Cote d'Azur' flowers (experiment M4). Flowers were pulsed with 0 (control), 200, 400 and 600 μM MeJA, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 3 days at 5, 12 or 20°C. Disease assessments were carried out daily for 3 succesive days after artificial inoculation. Data for independent treatment means are presented in Figure 4.8.

Factors	**Disease variables**		
	Disease severity (score 0-4) [a]	**Lesion number**	**Lesion diameter (mm)**
1) Temperature (°C) [b]			
5	0.7 a	13 a	0.64 a
12	2.3 b	36 b	0.84 c
20	2.3 b	39 b	0.75 b
2) Pulse MeJA concentration (μM)			
0	2.1 c	34 b	0.87 b
200	1.3 a	25 a	0.75 a
400	1.6 ab	25 a	0.71 a
600	1.8 bc	30 ab	0.69 a

[a] Data are main factor means of disease severity, lesion number and lesion diameter.
[b] Within main factor means, numbers followed by the same letter are not significantly different at P = 0.05.

At 12°C, freesia flowers treated with either 200 or 400 μM MeJA showed significantly ($P < 0.05$) reduced disease severity and lesion numbers compared to the untreated control (Figure 4.8; Appendix A4.2.4, Tables A4.2.4.4 and A4.2.4.6). However, at 20°C, only flowers treated with 200 μM MeJA showed significantly ($P < 0.05$) reduced disease severity and lesion numbers compared to the untreated control (Figure 4.8; Appendix A4.2.4, Tables A4.2.4.4 and A4.2.4.6).

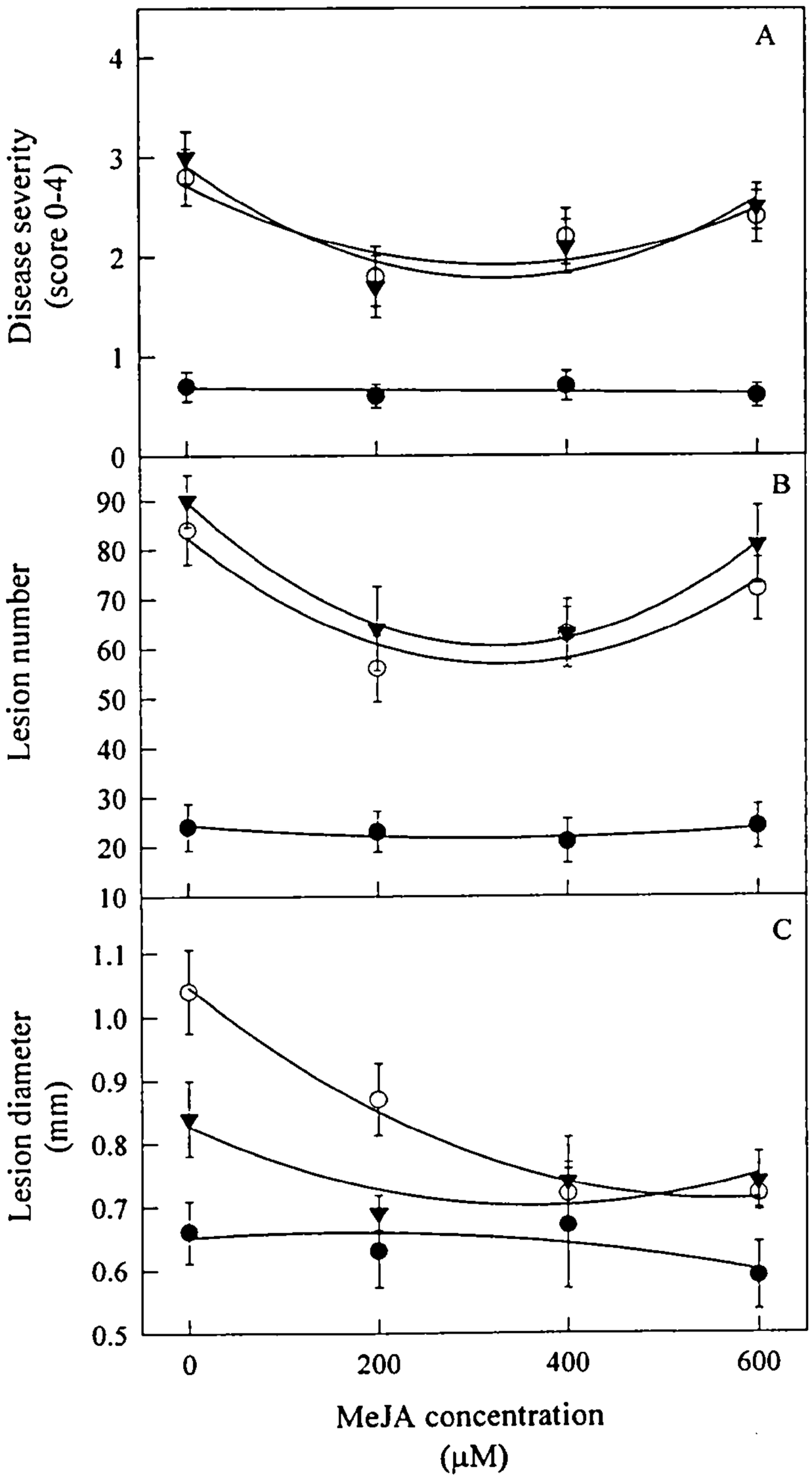

Figure 4.8: Quadratic regression of disease severity (A), lesion number (B) and lesion diameter (C) of freesia var. 'Cote d'Azur' flowers pulsed with 0 (control), 200, 400 and 600 μM MeJA, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5 (●), 12 (○) and 20°C (▼) (experiment M4). Each data point represents the mean data collected on 3 successive days of incubation. Bars indicate the SE for each treatment (n = 12). Main factor means are presented in Table 4.15 and regression parameters in Appendix 4.2.4, Table A4.2.4.9.

Treatment of cut freesia flowers with 600 μM MeJA had no effect on *B. cinerea* disease at any of the temperatures tested (Figure 4.8; Appendix A4.2.4, Tables A4.2.4.4, A4.2.4.6 and A4.2.4.8). Moreover, for incubation of MeJA treated freesia flowers at 5°C, there was no differential concentration effect on *B. cinerea* disease (Figure 4.8; Appendix A4.2.4, Tables A4.2.4.4, A4.2.4.6 and A4.2.4.8).

Lesion diameters on detached petals were significantly ($P < 0.05$) reduced in flowers treated with 400 and 600 μM compared to controls (Table 4.16; Appendix 4.2.4, Table A4.2.4.10). Treatment with 200 μM MeJA did not have any effect in reducing lesion diameter on detached petals compared to controls (Table 4.16).

In general, disease severity and lesion numbers were positively correlated with senescence as measured with the arbitary scale described in section 4.1.2.5 of freesias (Table 4.17). Disease severity and lesion numbers increased significantly ($P < 0.05$) with increasing flower senescence (Table 4.17). However, the diameter of lesions was not correlated with freesia senescence (Table 4.17).

Table 4.16: Lesion diameter on detached petals of freesia var 'Cote d'Azur' flowers pulsed with 0 (control), 200, 400 and 600 μM MeJA and incubated at 20°C for 48h (experiment M4).

Pulse MeJA concentration (μM)	Number of flower samples	Lesion diameter (mm) [a]
0	10	4.7 b
200	10	4.3 ab
400	10	3.9 a
600	10	3.6 a

[a] Within detached petal variable, numbers followed by the same letter are not significantly different at $P = 0.05$.

Table 4.17: Effect of senescence on disease severity, lesion numbers and lesion diameters of freesia var 'Cote d'Azur' flowers pulsed with MeJA, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 3 days at 5, 12 and 20°C (experiment M4).

Disease variable	**Number of observations**	**Senescence [a]**
Disease severity	320	0.737 **[b]
Lesion number	320	0.233 **
Lesion diameter	140	0.078 ns

[a] Senescence was measured according to the arbitary scale described in section 4.1.2.5.
[b] Data are results from Pearson's correlation at P = 0.05
** Significance at P = 0.05, ns: not significant at P = 0.05

4.2.3.5 Effect of pulse MeJA on non-inoculated (naturally infected) flowers (experiment M5)

Lesion numbers were significantly ($P < 0.05$) reduced on naturally infected freesia flowers incubated at 12°C irrespective of the MeJA concentration tested (Table 4.18; Appendix 4.2.5, Table A4.2.5.1). However, no significant ($P > 0.05$) reduction in lesion numbers on freesia var. 'Cote d'Azur' flowers was recorded at incubation temperatures of 5 or 20°C compared to untreated controls (Table 4.18).

Table 4.18: Effect of postharvest MeJA pulse treatment on non-inoculated (naturally infected) freesia var. 'Cote d'Azur' flowers (experiment M5). Flowers were pulsed with 0 (control), 200, 400, or 600 μM MeJA and incubated at 5, 12 and 20°C. Lesion number assessments were carried out daily. Each data point represents the mean data collected on 3 successive days of incubation.

Temperature (°C)	Pulse MeJA concentration (μM)	Lesion number [a]
5	0	3.3 ab
	200	3.3 ab
	400	2.1 a
	600	3.2 ab
12	0	7.1 c
	200	3.7 ab
	400	2.1 a
	600	3.2 ab
20	0	5.2 b
	200	3.7 ab
	400	3.6 ab
	600	3.4 ab

[a] For the MeJA by temperature interaction, numbers followed by the same letter are not significantly different at P = 0.05.

4.2.3.6 Effect of pulse MeJA on freesia vase life (experiment M6)

Relative fresh weight of untreated control freesia flowers was maintained greater from day 6 to day 10 of vase life compared to those treated with MeJA, irrespective of the concentration used (Figure 4.9B; Appendix 4.2.6, Tables A4.2.6.11–A4.2.6.17). Significantly ($P < 0.05$) higher wilt scores for flowers treated with 600 μM MeJA were observed on days 6, 7, 8 and 9 of incubation (Figure 4.9A; Appendix 4.2.6, Tables A4.2.6.1–A4.2.6.10).

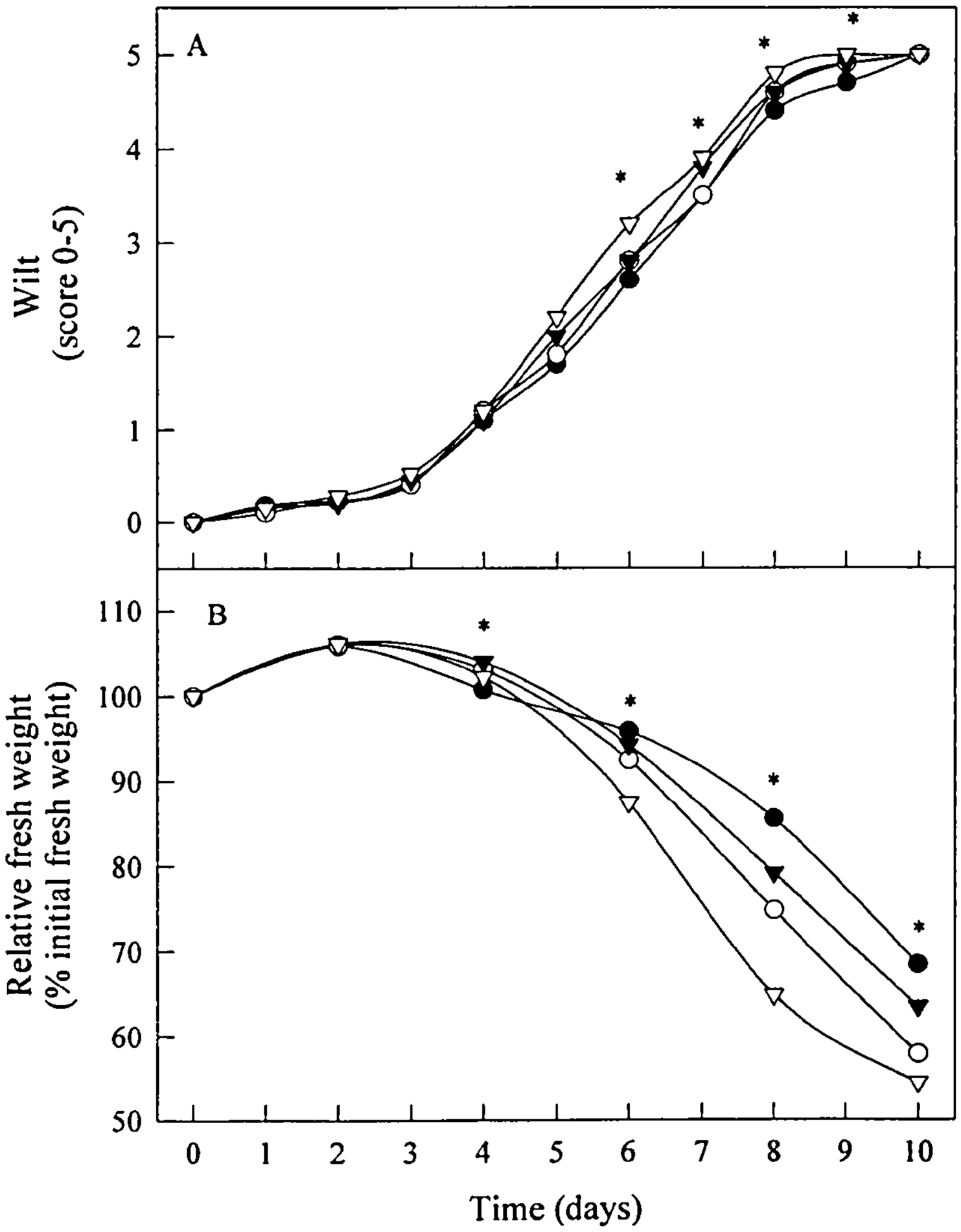

Figure 4.9: Wilt score (A) and relative fresh weight (B) of freesia flowers pulsed with 0 (●), 200 (○), 400 (▼) and 600 (▽) μM MeJA and incubated at 20°C (experiment M6). Stars indicate significant difference between treatments at P = 0.05 (Appendix 4.2.6, Tables A4.2.6.1-A4.2.6.17).

Vase life of freesia flowers treated with 400 or 600 μM MeJA was significantly ($P < 0.05$) reduced compared with untreated flowers (Table 4.19; Appendix 4.2.6, Table A4.2.6.18). Treatment of freesia flowers with 200 μM MeJA did not affect vase life (Table 4.19).

Table 4.19: Vase lives (days) of freesia var. 'Cote d'Azur' flowers pulsed with 0 (control), 200, 400 and 600 μM MeJA and incubated at 20°C (experiment M6).

MeJA concentration (μM)	Vase life (days) [a]
0	10.8 a
200	10.3 ab
400	10.2 b
600	9.5 b

[a] Within vase life variable, numbers followed by the same letter are not significantly different at P = 0.05.

4.2.3.7 Effect of MeJA applied as a spray on artificially inoculated freesia flowers (experiment M7)

Disease severity, lesion numbers and lesion diameters were MeJA concentration dependent (Table 4.20). Disease severity main factor means were significantly ($P < 0.05$) lower on flowers treated with 400 and 600 μM compared to untreated control flowers (Table 4.20). Although lesion numbers were significantly ($P < 0.05$) reduced after MeJA treatment at all concentrations lesion diameters were not reduced compared to untreated controls (Table 4.20). Temperature significantly affected disease severity, lesion numbers and lesion diameters (Table 4.20). Flowers incubated at 12°C showed significantly ($P < 0.05$) higher disease severity scores, lesion numbers and lesion diameters compared to those incubated at either 5 or 20°C (Table 4.20).

Table 4.20: Effect of postharvest methyl jasmonate (MeJA) treatment on *B. cinerea* disease suppression on freesia var. 'Cote d'Azur' flowers (experiment M7). Flowers were sprayed with 0 (control), 200, 400 and 600 µM MeJA, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 3 days at 5, 12 or 20°C. Disease assessments were carried daily for 3 successive days after artificial inoculation. Data for independent treatment means are presented in Figure 4.10.

Factors	Disease variables		
	Disease severity (score 0-4) [a]	Lesion number	Lesion diameter (mm)
1) Temperature (°C) [b]			
5	0.2 a	9 a	0.69 a
12	1.0 c	32 c	1.00 b
20	0.7 b	27 b	0.73 a
2) Spray MeJA concentration (µM)			
0	0.8 c	29 c	0.9 a
200	0.7 bc	23 b	0.9 a
400	0.6 ab	20 ab	0.7 a
600	0.5 a	19 a	0.8 a

[a] Data are main factor means of disease severity, lesion number and lesion diameter.
[b] Within main factor means, numbers followed by the same letter are not significantly different at P = 0.05.

Freesia flowers treated with 400 and 600 µM MeJA and incubated at 20°C showed significantly ($P < 0.05$) reduced disease severity and lesion numbers compared to untreated controls (Figure 4.10; Appendix 4.2.7, Table A4.2.7.4 and A4.2.7.6). Likewise, treatment of freesia flowers with 400 and 600 µM MeJA significantly ($P < 0.05$) reduced lesion numbers at 12°C (Figure 4.10; Appendix 4.2.7, Table A4.2.7.6). In addition, treatment with 200 and 600 µM MeJA significantly ($P < 0.05$) reduced lesion diameters at 20°C compared to untreated controls (Figure 4.10; Appendix 4.2.7, Table A4.2.7.8).

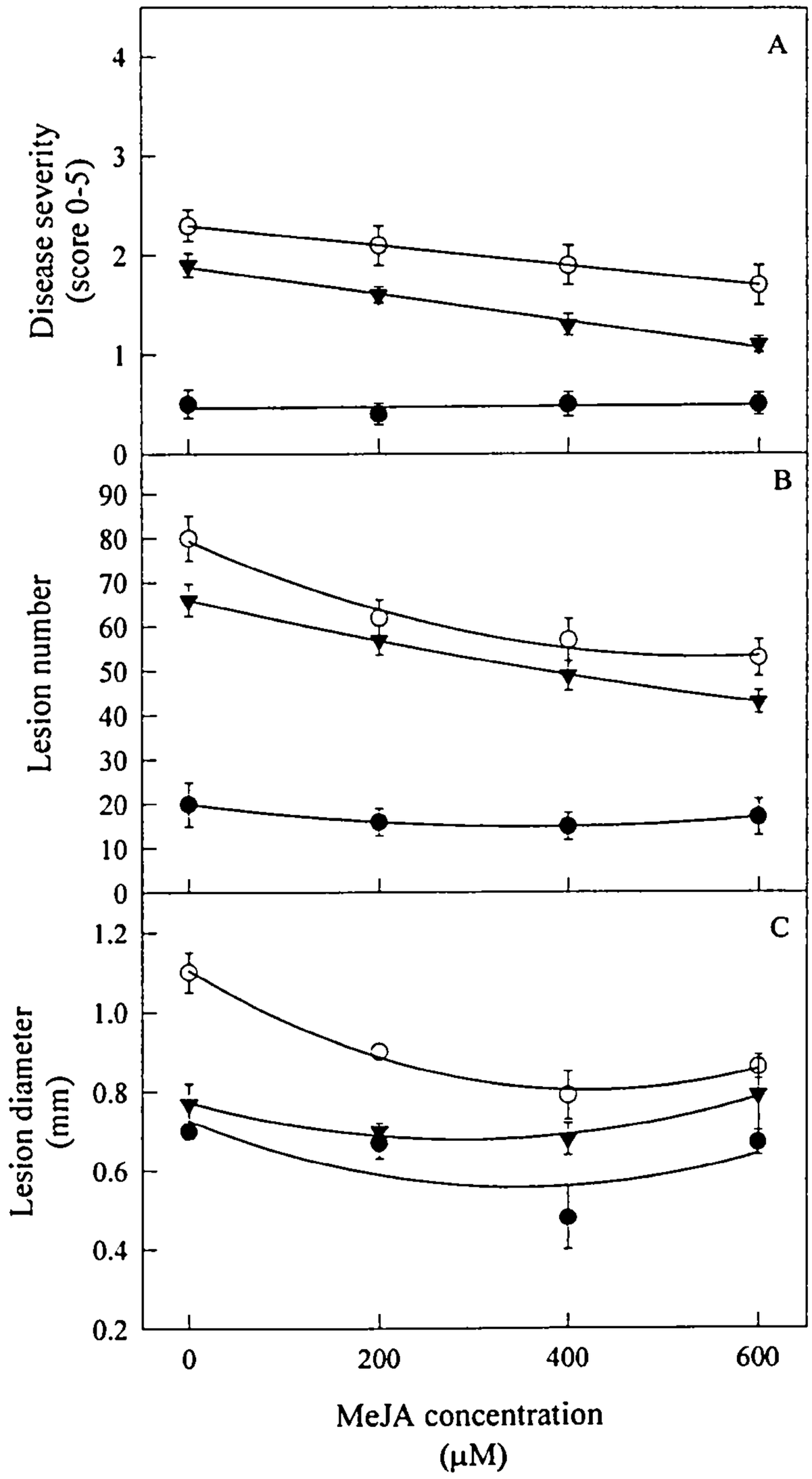

Figure 4.10: Quadratic regression of disease severity (A), lesion number (B) and lesion diameter (C) of freesia var. 'Cote d'Azur' flowers sprayed with 0 (control), 200, 400 and 600 μM MeJA, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5 (●), 12 (○) and 20°C (▼) (experiment M7). Each data point represents the mean data collected on 3 successive days of incubation. Bars indicate the SE for each treatment (n = 12). Main factor means are presented in Table 4.20 and regression parameters in Appendix 4.2.7, Table A4.2.7.9.

Lesion diameters on detached freesia petals were reduced by MeJA compared to untreated controls, but only upon treatment with 600 μM MeJA (Table 4.21; Appendix 4.2.7, Table A4.2.7.10). Positive correlations were found between disease severity and senescence, lesion number and senescence and between lesion diameters and senescence (Table 4.22).

Table 4.21: Lesion diameter on detached petals of freesia var 'Cote d'Azur' flowers sprayed with 0 (control), 200, 400 and 600 μM MeJA and incubated at 20°C for 48h (experiment M7).

Spray MeJA concentration (μM)	Number of flower samples	Lesion diameter (mm) [a]
0	10	4.6 b
200	10	4.0 ab
400	10	4.2 ab
600	10	3.7 a

[a] Within detached petal variable, numbers followed by the same letter are not significantly different at P = 0.05

Table 4.22: Effect of senescence on disease severity, lesion numbers and lesion diameters of freesia var 'Cote d'Azur' flowers sparyed with MeJA, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 3 days at 5, 12 and 20°C (experiment M7).

Disease variables	Number of observations	Senescence [a]
Disease severity	287	0.359**[b]
Lesion number	287	0.372**
Lesion diameter	128	0.201*

[a] Senescence was measured according to the arbitary scale described in section 4.1.2.5.
[b] Data are results from Pearson's correlation at P = 0.05
** Significance at P = 0.05, ns: not significant at P = 0.05

4.2.3.8 Effect of MeJA applied as a spray on non-inoculated flowers (experiment M8)

Significant ($P < 0.05$) reduction of lesion numbers were observed on freesia flowers treated with MeJA at all 3 rates compared to untreated control independently of incubation temperature (Table 4.23; Appendix 4.2.8, Table A4.2.8.1). Lesion number reduction on MeJA treated freesia flowers compared to controls was 50%, again independent of both MeJA concentration and incubation temperature (Table 4.23).

Table 4.23: Effect of postharvest MeJA spray treatment on non-inoculated (naturally infected) freesia var. 'Cote d'Azur' flowers (experiment M8). Flowers were sprayed with 0 (control), 200, 400, or 600 μM MeJA and incubated at 5, 12 and 20°C. Lesion number assessments were carried out daily. Each data point represents the mean data collected on 3 successive days of incubation.

Temperature (°C)	Spray MeJA concentration (μM)	Lesion number [b]
5	0	2.4 d
	200	0.8 abc
	400	0.8 abc
	600	0.7 abc
12	0	2.0 cd
	200	1.4 bcd
	400	1.1 abc
	600	1.1 abc
20	0	2.1 cd
	200	0.9 abc
	400	0.2 a
	600	0.5 ab

[a] For the MeJA by temperature interaction, numbers followed by the same letter are not significantly different at $P = 0.05$.

4.2.3.9 Effect of MeJA applied as a spray on freesia vase life (experiment M9)

Relative fresh weight of MeJA treated flowers was maintained significantly ($P < 0.05$) higher compared to that of the untreated controls (Figure 4.11 B; Appendix 4.2.9, Tables A4.2.9.11–A4.2.9.16).

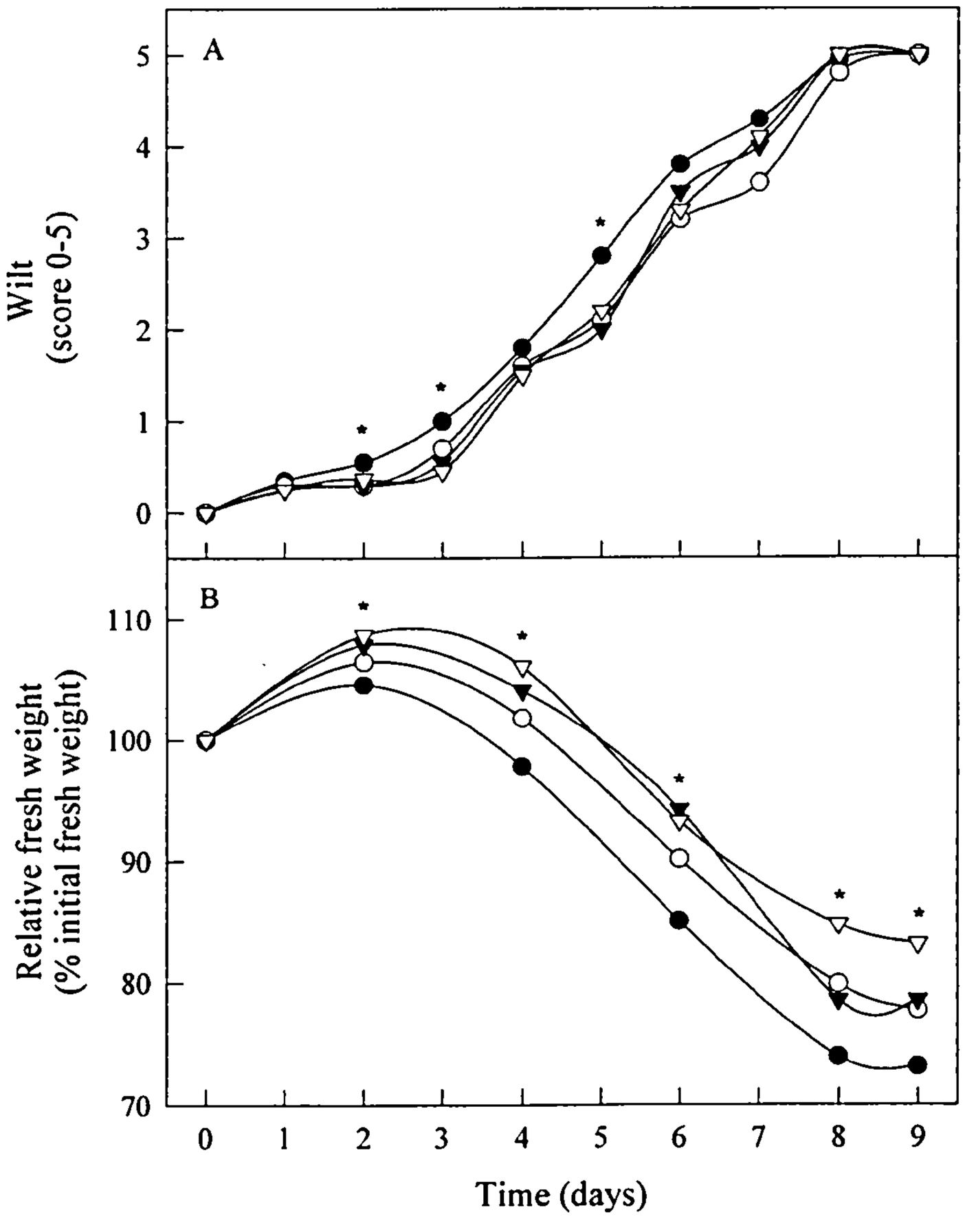

Figure 4.11: Wilt score (A) and relative fresh weight (B) of freesia flowers sprayed with 0 (●), 200 (○), 400 (▼) and 600 (▽) μM MeJA and incubated at 20°C (experiment M9). Stars indicate significant difference between treatments at $P = 0.05$ (Appendix 4.2.9, Tables A4.2.9.1-A4.2.9.16).

Moreover, at 20°C, wilt scores on days 2, 3 and 5 of incubation were significantly ($P < 0.05$) lower in MeJA treated flowers than in the untreated control (Figure 4.11 A; Appendix 4.2.9, Tables A4.2.9.1–A4.2.9.10). Vase lives of freesia flowers treated with 200, 400 or 600 μM MeJA and incubated at 20°C were not significantly ($P > 0.05$) different to that of the untreated control (Table 4.24; Appendix 4.2.9, Table A4.2.9.17).

Table 4.24: Vase lives (days) of freesia var. 'Cote d'Azur' flowers sprayed with 0 (control), 200, 400 and 600 μM MeJA and incubated at 20°C (experiment M9).

MeJA concentration (μM)	Vase life (days) [a]
0	10.2 a
200	10.4 a
400	10.0 a
600	9.8 a

[a] Within vase life variable, numbers followed by the same letter are not significantly different at $P = 0.05$.

4.2.3.10 Effect of MeJA on mycelial growth, conidial germination and germ tube elongation (experiment M10)

In-vitro mycelial growth of *B. cinerea* isolate (BcF1) on ½ strength PDA was significantly ($P < 0.05$) less on media supplemented with 600 μM MeJA compared to untreated controls (Figure 4.12; Appendix 4.2.10, Table A4.2.10.3). MeJA treatments at 200, 400 and 600 μM facilitated *B. cinerea* conidial germination. Also, MeJA at 200, 400 and 600 μM did not affect *B. cinerea* germ tube elongation (Table 4.25; Appendix 4.2.10, Tables A4.2.10.1 and A4.2.10.2, respectively).

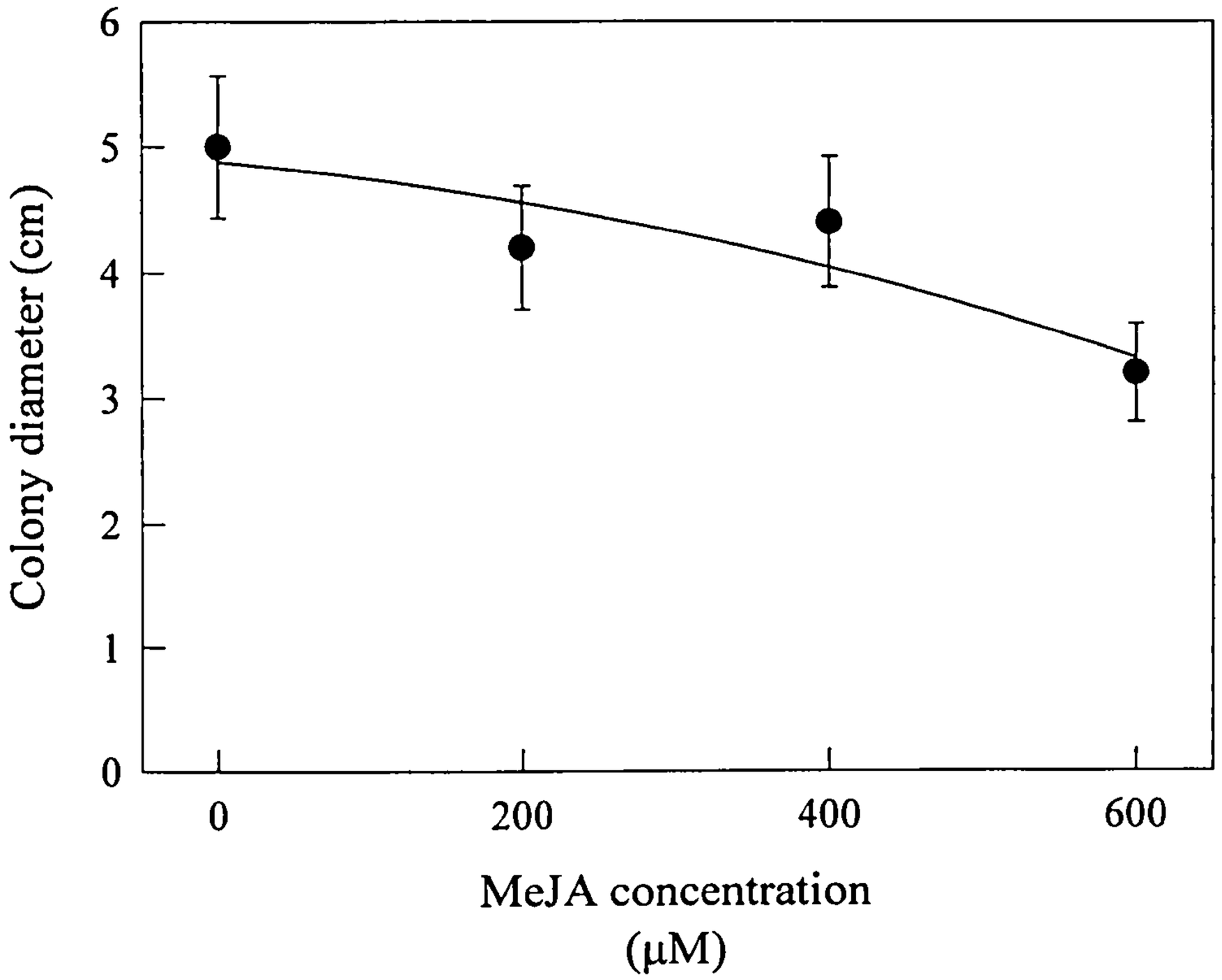

Figure 4.12: *In-vitro* mycelial growth of *B. cinerea* isolate (BcF1) on ½ PDA supplemented with 0, 200, 400 and 600 μM MeJA. Data are means of a 5-day incubation period at 20°C under UV-A light (12h photoperiod). Regression line: $y = 4.88 - 0.0011x$, $R^2 = 0.83$. Bars indicate the SE (n = 25).

Table 4.25: Percent of *B. cinerea* conidial germination and germ tube elongation on media supplemented with 0, 200, 400 or 600 μM MeJA Conidial suspensions were incubated for 12h at 20°C in the dark.

MeJA concentration (μM)	***B. cinerea* conidial germination (%)** [a]	**Germ tube elongation (μm)**
0	74 a	45.9 a
200	90 b	41.2 a
400	93 b	51.5 a
600	94 b	49.6 a

[a] Within each variable, numbers followed by the same letter are not significantly different at P = 0.05.

4.2.4 Discussion

MeJA was generally effective in suppressing *B. cinerea* on cut freesia flowers. Its effectiveness was largely dependant on the mode of application and concentration. Gaseous MeJA was more effective compared to pulsing or spraying. In *Arabidopsis* plants, MeJA was also more effective in suppressing *B. cinerea* when applied as gas rather than as spray (Thomma *et al.*, 2000). The results of the present study also show that pulse treatment of freesia flowers with MeJA reduced *B. cinerea* disease severity, but only at 200 μM concentration. These results agree with those of Meir *et al.* (1998), where disease severity in both artificially inoculated and naturally infected rose flowers was significantly reduced by a MeJA pulse at 200 μM. However, the degree of protection varied between cultivars (Meir *et al.*, 1998). Spray application, especially at 600 μM MeJA, significantly reduced disease severity and lesion number on cut freesia flowers. Similarly, Thomma *et al.* (2000) found that spray MeJA application on *Arabidopsis* plants suppressed *B. cinerea*.

In the present study MeJA did not show any direct *in-vitro* antimicrobial effect. This suggests that MeJA reduced disease on freesia flowers by an induced response. Application of exogenous JA can induce a set of defence genes or compounds that are also activated upon pathogen infection (Terras *et al.*, 1995; Epple *et al.*, 1997). These genes include those encoding plant defensins and thionins (Terras *et al.*, 1995; Epple *et al.*, 1997), phytoalexins (Il'inskaya *et al.*, 1996; Nojiri *et al.*, 1996), PR-proteins (Xu *et al.*, 1994) and proteinase inhibitors (Thaler *et al.*, 1996; Creelman and Mullet, 1997).

The effect of MeJA was also temperature dependent in that it gave better disease suppression at 20°C than at 5 or 12°C. There is no published work on how temperature affects gene regulation or antimicrobial compound production in plant tissue. Based on the results of all of MeJA experiments, it was indicated that low (5°C) or high (20°C) incubation temperatures affected considerably MeJA activity. These findings contradict a previous study on grapefruit where low incubation temperatures (2°C) did not affect MeJA efficacy against *Penicilium digitatum* on grapefruit (Droby *et al.*, 1999).

Suppression of *B. cinerea* on cut flowers with pulse MeJA was firstly reported by Meir *et al.* (1998). In the present study it was demonstrated that MeJA may also be used as postharvest treatment via gaseous application mode to suppress *B. cinerea* on cut

freesia flowers. In the context of an environmental friendly disease management strategy, the natural product MeJA can provide a marked level of protection thereby offering an alternative to synthetic chemical fungicides.

4.3 FURTHER ASSESSMENT OF POSTHARVEST TREATMENT WITH GASEOUS METHYL JASMONATE ON INDUCED DEFENCE RESPONSES

4.3.1 Introduction

Gaseous methyl jasmonate protected SA-degrading transformant *NahG Arabidopsis* plants (Thomma *et al.*, 2000). This protection suggests that MeJA treatment induced a non-SA dependent systemic response. Treatment of *Arabidopsis* plants with gaseous MeJA resulted in reduced *B. cinerea* disease severity. Moreover, gaseous MeJA application resulted in greater disease reduction compared to plants sprayed with MeJA or INA (Thomma *et al.*, 2000).

The polyphenol oxidase (PPO) enzyme oxidizes phenolic compounds to quinones. Quinones are reactive molecules that interact with many other biological molecules (Constabel and Ryan, 1998). For example, they are toxic to herbivores and to fungal and bacterial pathogens (Taiz and Zeiger, 1998). MeJA is a compound closely related to components of the octadecanoid pathway, and is a strong inducer of PPO (Constabel and Ryan, 1998).

Phenylalanine is a common amino acid produced via the shikimic pathway. The PAL enzyme catalyses the production of SA from phenylalanine (Sticher *et al.*, 1997). Complex cross-talking between SA and JA/ethylene pathways has been documented (Glazebrook 2001). In many cases, SA can down-regulate the JA-dependant pathway (Pena-Cortes *et al.*, 1993; Lawton *et al.*, 1994).

The aim of this experiment was to investigate the effect of gaseous MeJA at 0.1 $\mu L\ L^{-1}$ over time on infection over time of freesia var. 'Cote d'Azur" flowers by *B. cinerea* PAL and PPO activities were measured.

4.3.2 Materials and methods

4.3.2.1 Plant material

Freesia flowers var 'Cote d' Azur' were provided by Zwetsloots & Sons Ltd (UK) (Appendix 2.2, Plate A2.1). Flowers were at the commercial harvest stage with all buds still closed (Appendix 2.2, Plate A2.2,). They were processed in the laboratory approximately 24h after harvest.

4.3.2.2 Experiment design

In MeJA gaseous experiments Mg1 and Mg2, 10 replicate flowers per treatment were either artificially inoculated with a 10^4 *B. cinerea* conidial mL^{-1} suspension (experiment Mg1) or left non-inoculated (experiment Mg2) (Table 4.26). In these experiments, factors were time (0, 6, 12, 24 and 48h) and chemical treatment (0 and 0.1 $\mu L\ L^{-1}$ MeJA). In the MeJA biochemical analysis experiments Mg3 and Mg4, 3 replicates of 3 sample flowers (i.e. corollas) each (7-10 g FW^{-1}) were used.

Table 4.26: Experiment number, freesia variety, number of replications, inoculum treatment concentration, assessment parameters and other treatment variables pertaining in the gaseous MeJA experiments.

Experiment	Replications	Assessments [a]	Factors [b]
Mg1	10	Disease	Ti x Mg
Mg2	10	Disease	Ti x Mg
Mg3	3 x 3	PPO Assay	Ti x Mg x I
Mg4	3 x 3	PAL Assay	Ti x Mg x I

[a] Disease assessments include disease severity, lesion number and lesion diameter.
[b] Mg: MeJa gaseous treatment, I: Inoculum, Ti: Time.

4.3.2.3 Chemical treatments, inoculation and incubation

MeJA gaseous treatments were performed in gas-tight PVC containers (60 x 60 x 90 cm). MeJA gas concentrations were calculated based on the assumption that MeJA completely evaporated. The treatment concentrations were 0 (control) and 0.1 μL MeJA L^{-1}. Twenty-four hours after MeJA gaseous treatment, flowers were either artificially inoculated or left non-inoculated. Assessments of disease parameters and PAL and PPO activity were made 0, 6, 12, 24 and 48h following MeJA treatment.

4.3.2.4 Artificial inoculation

Artificial inoculation of flowers with *B. cinerea* conidial suspensions was carried out as described in section 3.2.2.2. The conidial concentration was adjusted to 10^4 *B. cinerea* conidia mL^{-1} with a haematocytometer using two counts per conidial suspension.

4.3.2.5 PPO activity

The effect of gaseous MeJA on PPO activity of freesia var. 'Cote d'Azur' flowers was investigated in experiment Mg3. PPO was measured according to the methods of Martinez-Tellez and Lafuente (1993). Weighed flowers were snap frozen and ground in liquid nitrogen. The resulting tissue powder was added to ice cold 0.05 M potassium phosphate buffer, pH 7.2, containing 1 M KCl (3 mL g FW^{-1}). PPO was extracted by gentle stirring for 1h at 4°C. After stirring, the solution was filtered through a Whatman filter paper No. 1 and centrifuged at 20,000 x g for 10 min at 4°C (Martinez-Tellez and Lafuente, 1993).

Enzyme activity was determined by measuring the rate of increase in absorbance at 410 nm of the reaction mixture at room temperature (*ca.* 23°C). The reaction mixture contained 2.5 mL 0.02 M caffeic acid (Acros Organics, UK) and 0.5 mL of the supernatant. One unit of PPO activity was defined as an increase of 0.001 unit of

absorbance per min at 410 nm. PPO activity was expressed as units of PPO g FW^{-1} min^{-1} (Martinez-Tellez and Lafuente, 1993).

4.3.2.6 PAL activity

The effect of gaseous MeJA in PAL activity of freesia var. 'Cote d'Azur' flowers was investigated in experiment Mg4. PAL activity was measured as described in section 4.1.2.6.

4.3.2.7 Assessments

Disease severity score, lesion numbers, lesion diameters and senescence were recorded on artificially inoculated flowers (experiment Mg1). Disease severity of artificially inoculated flowers was evaluated daily using the arbitrary scale described in section 3.2.2.4. Lesion diameters of 10 randomly selected lesions per flower were measured daily after artificial inoculation as described in section 3.2.2.4. In gaseous MeJA experiment Mg2, lesion numbers were recorded on non-inoculated (naturally infected) flowers.

4.3.2.8 Statistical analysis

Data from experiments Mg1-Mg4 were analysed as described in section 4.1.2.8 using SPSS 9.0 for Windows. Graphic representation in all experiments were performed using Sigmaplot 2000 for Windows. Data in text are presented as individual treatment means in figures.

4.3.3 Results

4.3.3.1 Effect of gaseous MeJA on artificially inoculated flowers (experiment Mg1)

In examining the effect of gaseous MeJA at 0.1 μL L^{-1} over a period of 48h of incubation at 20°C in the dark on disease severity, lesion numbers and lesion diameters the first disease symptoms on artificially inoculated flowers were observed after 12h (Figure 4.13). Disease symptoms on artificially inoculated flowers treated with MeJA were significantly ($P < 0.05$) lower compared to untreated control flowers throughout the 48h period of incubation (Figure 4.13 A, B, C; Appendix 4.3.1, Tables A4.3.1.4, A4.3.1.6 and A4.3.1.8).

Greatest differences in these disease pameters between MeJA treated flowers and the untreated control were recorded after 12h of incubation (Figure 4.13 A, B, C). At this time, disease severity, lesion number and lesion diameter of MeJA treated flowers were reduced by up to 68, 56 and 50%, respectively, compared to the untreated control (Figure 4.13 A, B, C). Thereafter, these symptoms on MeJA treated flowers were maintained significantly ($P < 0.05$) lower compared to untreated control flowers (Figure 4.13 A, B, C; Appendix 4.3.1, Tables A4.3.1.4, A4.3.1.6 and A4.3.1.8).

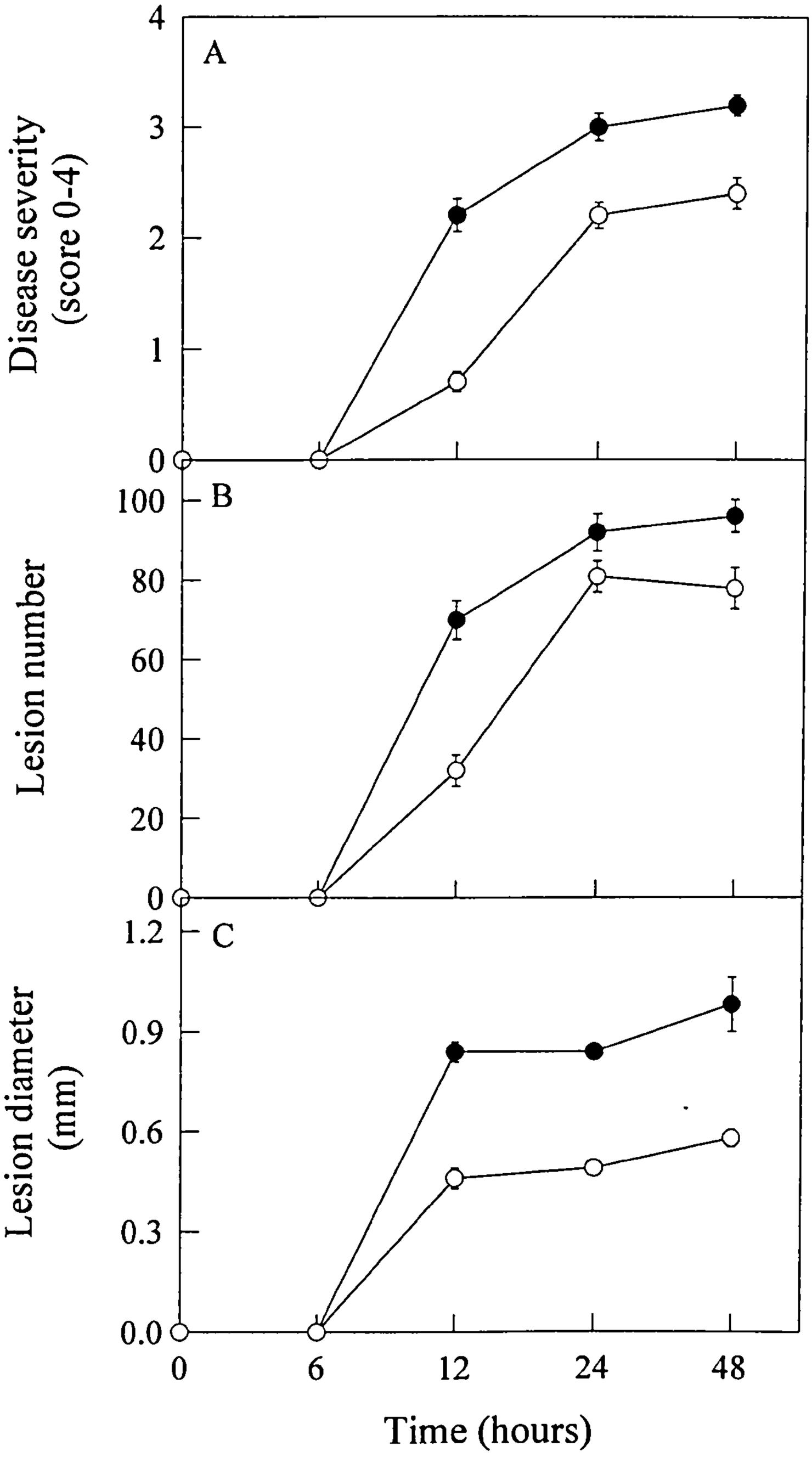

Figure 4.13: Effect of gaseous MeJA treatment on disease severity (A), lesion number (B) and lesion diameter (C) of freesia var. 'Cote d'Azur' flowers treated with 0.1 μL L^{-1} MeJA (○) or left untreated (controls) (●). The flowers were inoculated at 0h with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 48h in the dark. Bars indicate the SE for each treatment (n = 10).

4.3.3.2 Effect of gaseous MeJA on non-inoculated (naturally infected) flowers (experiment Mg2)

Significant ($P < 0.05$) reductions in lesion numbers on non-inoculated (naturally infected) freesia flowers were observed after 6, 12, 24 and 48h of incubation at 20°C (Figure 4.14; Appendix 4.3.2, Table A4.3.2.2).

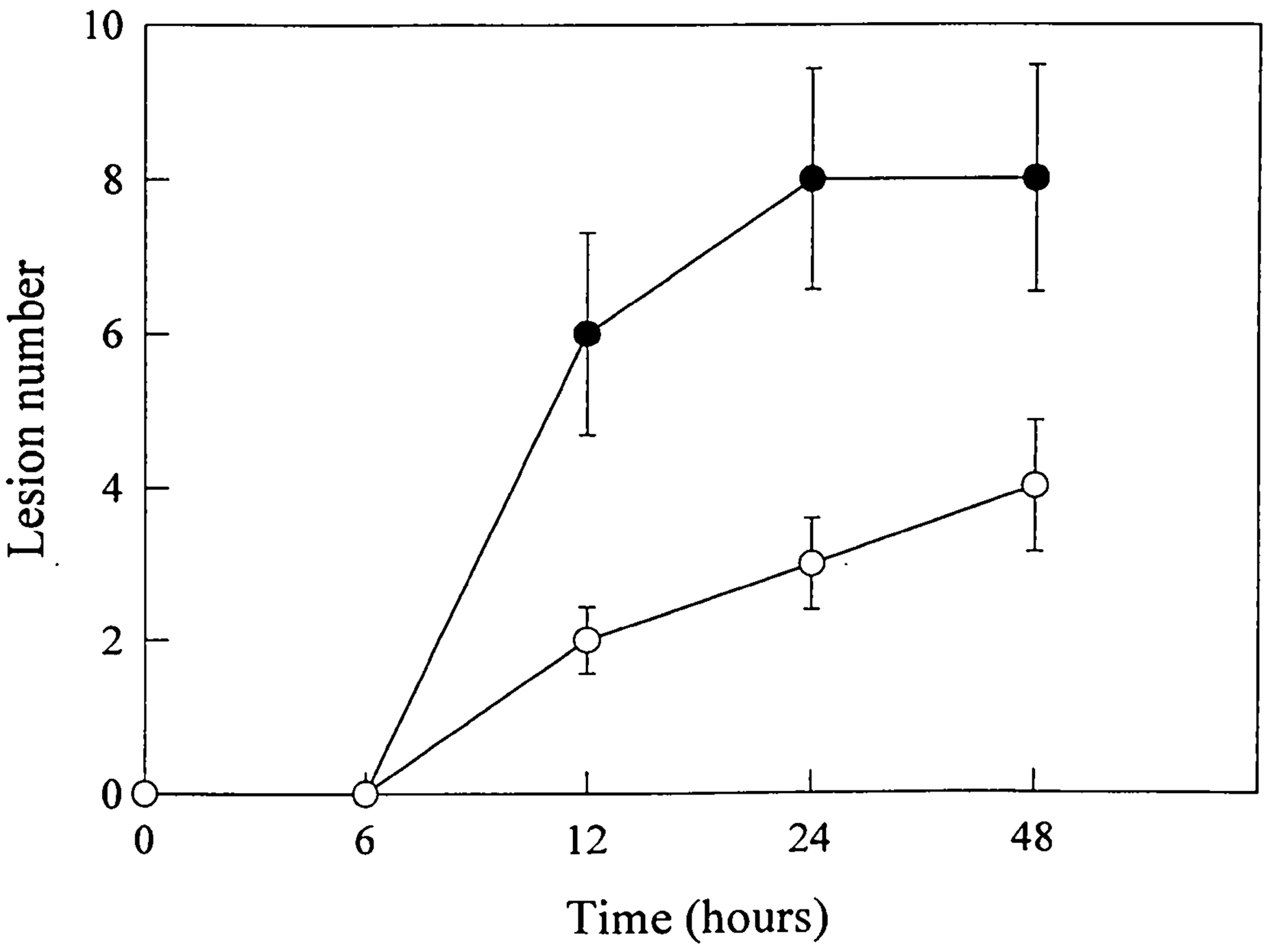

Figure 4.14: The effect of gaseous MeJA treatment on lesion number of non-inoculated (naturally infected) freesia var. 'Cote d'Azur' flowers. The flowers were treated with 0.1 $\mu L\ L^{-1}$ MeJA (○) or left untreated (control) (●). Bars indicate the SE for each treatment (n = 10).

4.3.3.3 PPO activity in gaseous MeJA treated flowers (experiment Mg3)

Variable PPO activity was manifest as 2 peaks in non-inoculated flowers and 1 peak in artificially inoculated flowers (Figure 4.15). The highest PPO values were recorded at 0h (i.e. 24h after MeJA treatment) for MeJA treated flowers compared to untreated controls (Figure 4.15; Appendix 4.3.3, Table A4.3.3.2). In non-inoculated MeJA treated flowers the second peak occurred after 12h of incubation (Figure 4.15).

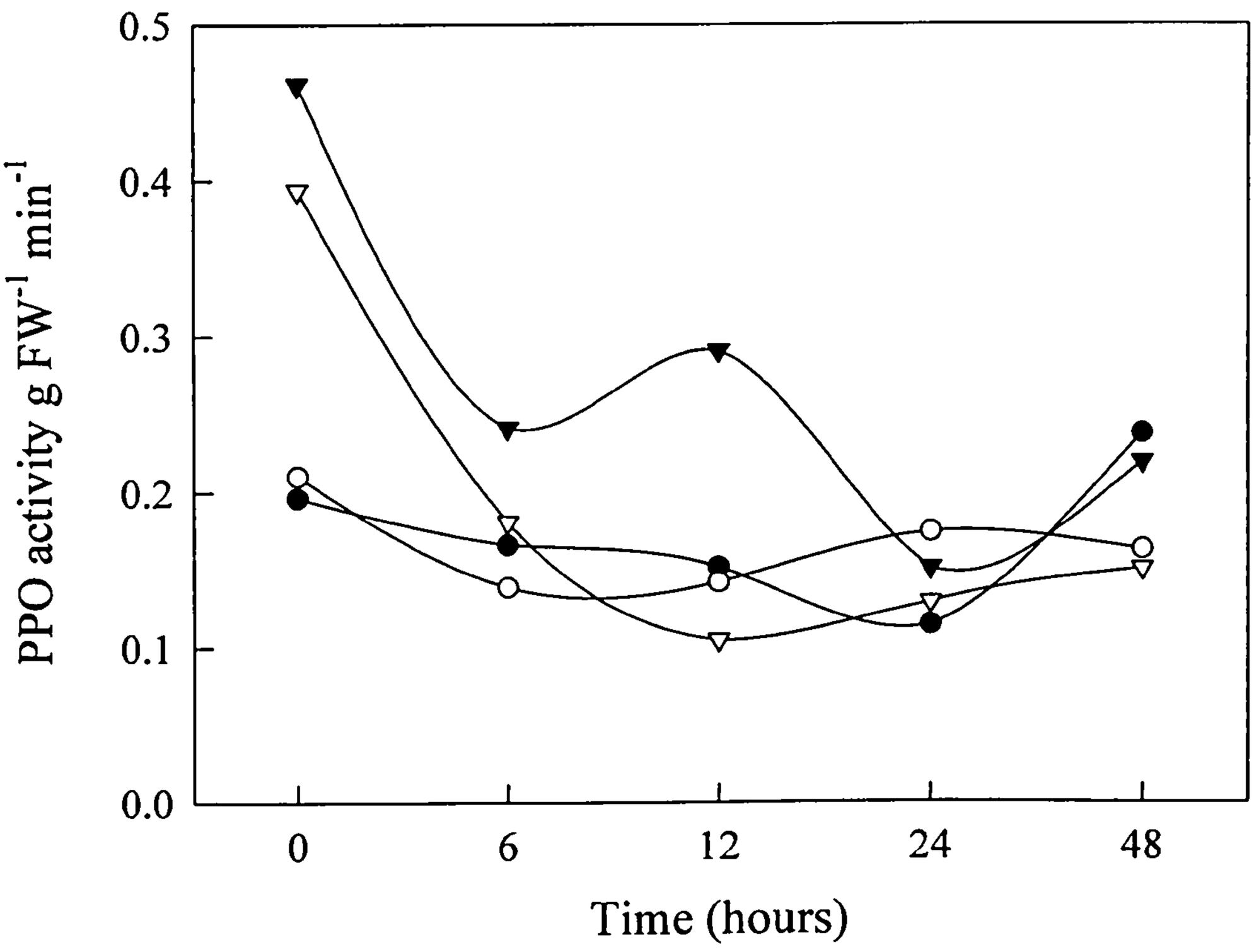

Figure 4.15: PPO activity of freesia var. 'Cote d'Azur' flowers treated with 0.1 μL MeJA L^{-1} and either artificially inoculated with 10^4 *B. cinerea* conidia mL^{-1} (▽) or left non-inoculated (▼). Untreated flowers that were artificially inoculated (○) or left non-inoculated (●) and served as control. SEM = 0.017 (n = 3).

4.3.3.4 PAL activity in gaseous MeJA treated flowers (experiment Mg4)

PAL activity was maintained at similar levels in non-inoculated treated and untreated flowers (Figure 4.16). In contrast, artificially inoculated control flowers showed a significantly ($P < 0.05$) higher PAL activity after 12, 24, and 48h of incubation (Figure 4.16; Appendix 4.3.4, Table A4.3.4.2). PAL levels of MeJA treated, artificially inoculated flowers were negligible after 12, 24, and 48h of incubation (Figure 4.16).

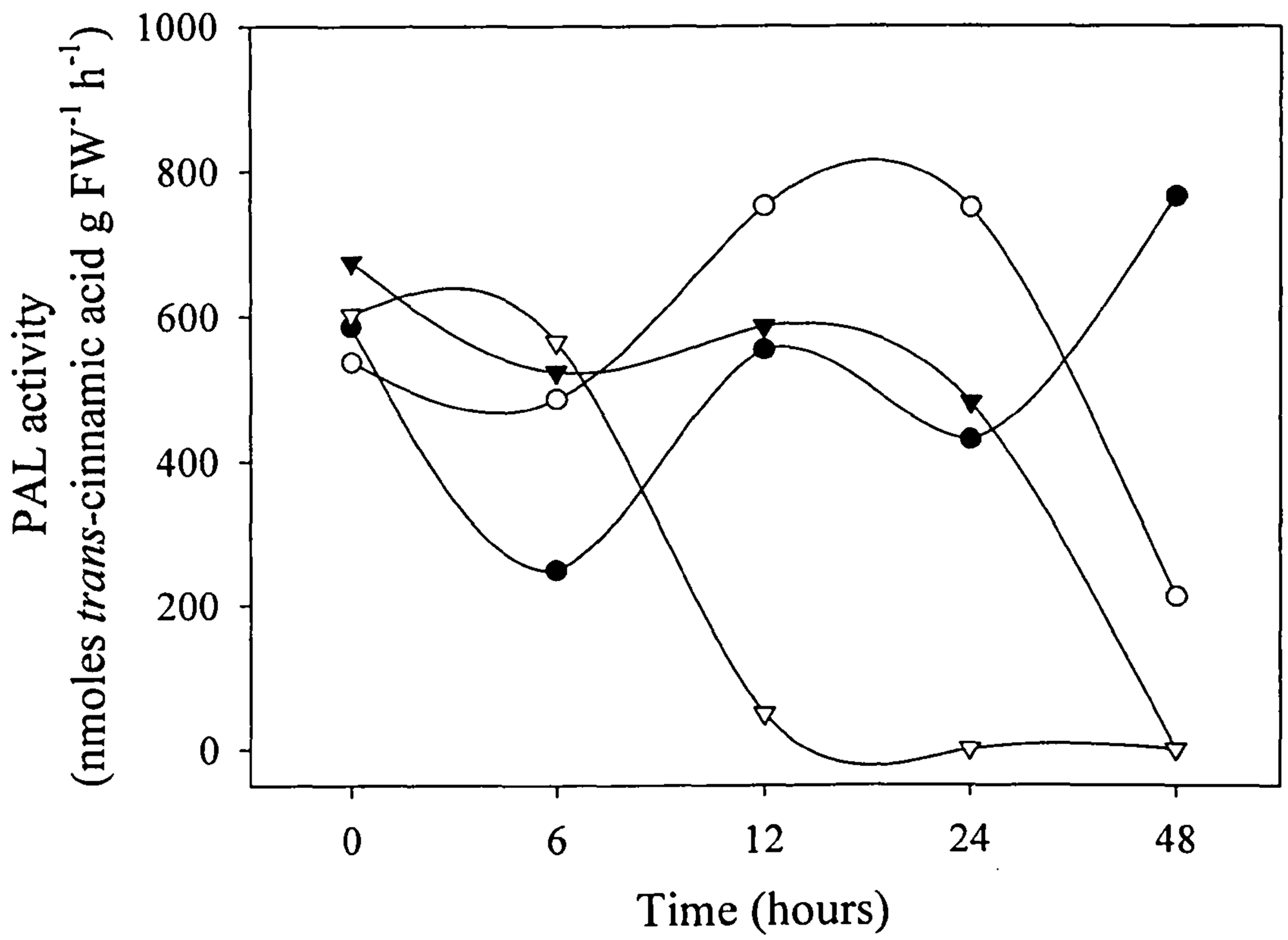

Figure 4.16: PAL activity of freesia var. 'Cote d'Azur' flowers treated with 0.1 μL MeJA L^{-1} and either artificially inoculated with 10^4 *B. cinerea* conidia mL^{-1} (▽) or left non-inoculated (▼). Untreated flowers that were artificially inoculated (○) or left non-inoculated (●) and served as control. SEM = 33.19 (n = 3).

4.3.4 Discussion

Increased PPO activity in freesia flowers was induced just after the 24h MeJA treatment (at the 0h of sampling). This observation is in agreement to previous research (Thaler *et al.*, 1996; Constabel and Ryan, 1998). PPO levels in freesia flowers decreased concomitant with senescence over time. This phenomenon is probably due to reduction in enzyme activity during tissue maturation that results in decreased disease resistance (Sommer, 1985).

PAL activity in untreated control freesia flowers was induced following artificial inoculation with *B. cinerea.* In contrast, PAL activity in MeJA treated freesia flowers was decreased significantly compared to untreated control 12h post-inoculation and thereafter. This trend was more pronounced when MeJA treated flowers were artificially inoculated with *B. cinerea.* PAL inhibition induced by ethylene has been recorded in grapefruit following their inoculation with *Penicillium digitatum* (Lisker *et al.*, 1983). These findings may suggest that MeJA treatment suppresses the action of PAL in driving the phenylpropanoid pathway and consequently blocking downstream SA production (Figure 2.4). Antagonistic regulation of JA- and SA-dependent pathways is well documented (Pena-Cortes *et al.*, 1993; Conconi *et al.*, 1996; Niki *et al.*, 1998; Gupta *et al.*, 2000; Rao *et al.*, 2000). Accordingly, in most cases up-regulation of one inter-dependent pathway will down-regulate another (Pena-Cortes *et al.*, 1993; Conconi *et al.*, 1996; Niki *et al.*, 1998; Gupta *et al.*, 2000; Rao *et al.*, 2000). Katz *et al.* (1998) demonstrated the effect of acibenzolar on PAL activity whereby PAL mRNA was induced in acibenzolar treated parsley cells in the presence of *Phytophthora megasperma* f. sp. *glycinea* (Katz *et al.*, 1998). Suppression of PAL by MeJA treatment might, therefore, be additional evidence of JA- and SA- antagonistic effect.

4.4 EFFECTS OF COMBINED TREATMENT OF ACIBENZOLAR-S-METHYL AND METHYL JASMONATE

4.4.1 Introduction

Cross talk between SA- and MeJA/ethylene-dependent pathways has been demonstrated in the past (Pena-Cortes *et al.*, 1993; Lawton *et al.*, 1994; Niki *et al.*, 1998; Gupta *et al.*, 2000). A combination of acibenzolar and JA treatments was tested on tomato field grown plants against bacterial and insect attack (Thaler *et al.*, 1999). The two signaling pathways, one involving SA and another involving JA were suggested to provide resistance against pathogens and insect herbivores, respectively (Thaler *et al.*, 1999). Combination of these two chemical activators as postharvest treatments for ornamentals have apparently not been tested.

Acibenzolar alone or in combination with different modes of MeJA application were examined. This was done with a view to induce both SA- and JA-dependant responses against *B. cinerea* on freesia flowers.

4.4.2 Materials and Methods

4.4.2.1 Plant material

Freesia flowers var 'Cote d' Azur' were provided by Zwetsloots & Sons Ltd (UK) (Appendix 2.2, Plate A2.1). Flowers were at the commercial harvest stage with all buds still closed (Appendix 2.2, Plate A2.2,). They were processed in the laboratory approximately 24h after harvest.

4.4.2.2 Experiment design

Ten replicate flowers per treatment were artificially inoculated with a 10^4 *B. cinerea* conidial mL^{-1} suspension and used for disease assessments. The experiment was arranged inside controlled temperature incubation rooms in a CRB design. Experiment was two-factor design with temperature (12 and 20°C) and chemical treatments as factors.

4.4.2.3 Chemical treatments

MeJA and acibenzolar were used in combination to investigate potential induced synergistic or antagonistic effects. MeJA and acibenzolar were prepared by dissolving both chemicals together at appropriate concentrations in distilled water. MeJA was applied in gaseous, pulse or spray form as described in section 4.2.2.4, either alone or in combination with 0.15 g AI L^{-1} acibenzolar. MeJA application in gaseous form was at 0.1 μL L^{-1}, in pulse form was at 200 μM and in spray form was at 600 μM. Treated freesia flowers were kept for 24h at 20°C in the dark.

4.4.2.4 Artificial inoculation

Twenty-four hours following chemical treatment, flowers were artificially inoculated with a suspension of 10^4 *B. cinerea* conidia mL^{-1} as described in section 3.2.2.2. The flowers were then incubated at 12 or 20°C and *ca.* 100% RH in the dark. The concentration of conidial suspensions was adjusted to 10^4 *B. cinerea* conidia mL^{-1} with a haematocytometer using two counts per conidial suspension.

4.4.2.5 Assessments

Disease

Disease severity score, lesion numbers and lesion diameters were recorded from artificially inoculated flowers. Disease severity was recorded daily using the arbitrary scale described in section 3.2.2.4. Lesion diameters of 10 randomly selected lesions per flower were measured each day following artificial inoculation done as described in section 3.2.2.4.

Detached petal bioassays

Lesion diameter on detached freesia petals was recorded according to the method described by Meir *et al.* (1998) and measured as described in section 4.1.2.5.

4.4.2.6 Statistical analysis

Data were analysed as described previously in section 4.1.2.8. Data are presented in individual treatment means in figure or table. The results of the statistical tests are presented in appendices.

4.4.3 Results

Freesia flowers treated with either gaseous MeJA at 0.1 μL L^{-1} and in combination with acibenzolar showed marked ($P < 0.05$) reduction of disease severity, lesion numbers and lesion diameters at 12°C and 20°C compared to all other chemical treatments (Figure 4.17 A, B, C; Appendix 4.4, Tables A4.4.4, A4.4.6 and A4.4.8). Disease severity, lesion numbers and lesion diameters were reduced by 54, 54 and 56%, respectively, on flowers treated with gaseous MeJA at 0.1 μL L^{-1} and incubated at 20°C compared to untreated controls (Figure 4.17 A, B, C).

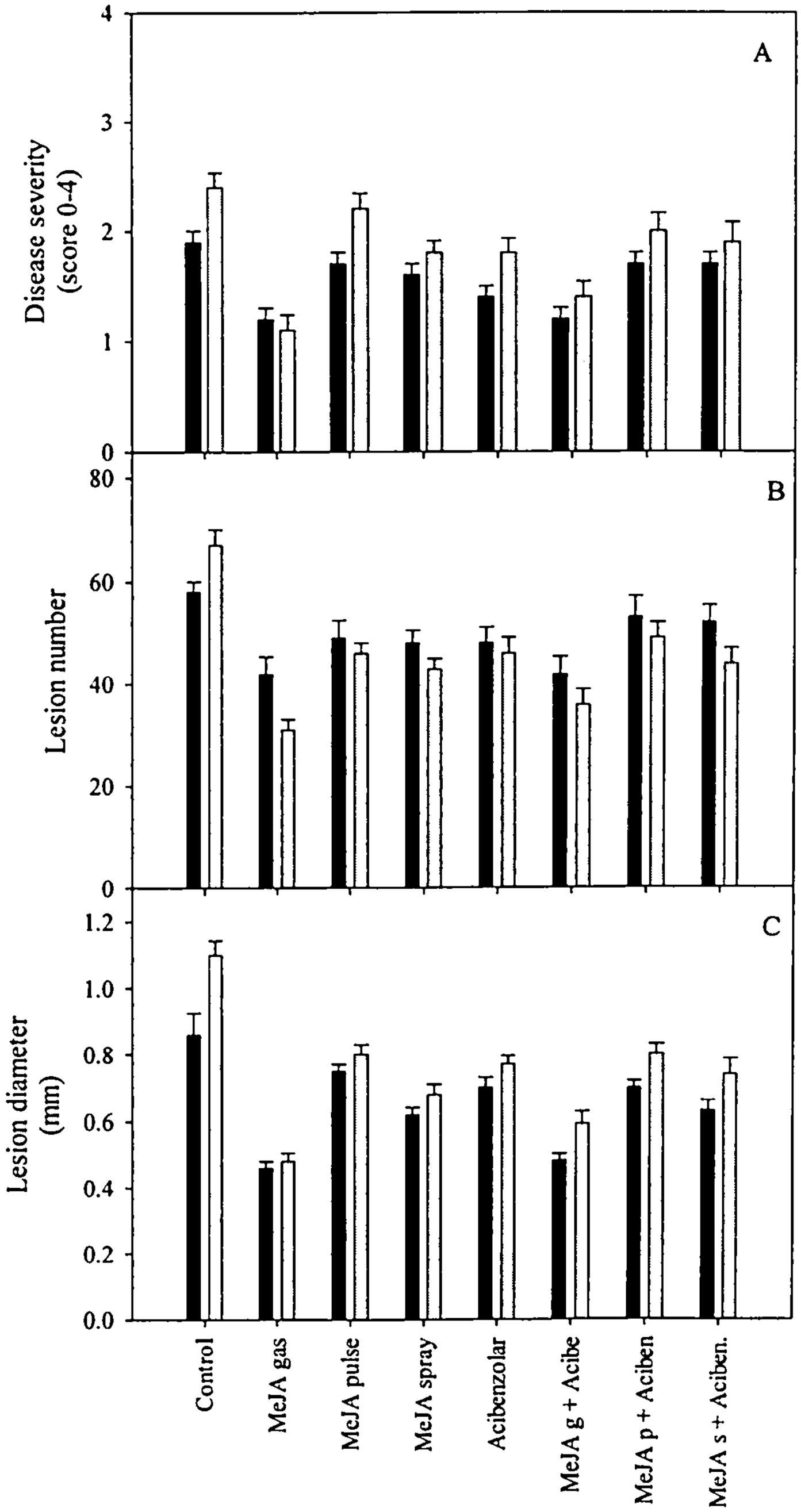

Figure 4.17: Disease severity (A), lesion number (B) and lesion diameter (C) of MeJA-acibenzolar combined postharvest experiment. The flowers were treated with MeJA by three application modes of gas 0.1 μL L^{-1}), pulse (200 μM) and spray (600μM) either alone or in combination with acibenzolar (0.15 g AI L^{-1}), inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 3 days at 12 (■) or at 20°C (▨) in the dark. Bars indicate the SE (n = 30).

Likewise at 12°C, disease severity, lesion number and lesion diameter were reduced by 37, 28 and 47%, respectively on flowers treated with gaseous MeJA at 0.1 μL L^{-1} and incubated at 20°C compared to controls (Figure 4.17 A, B, C). No synergistic effect after combined MeJA and acibenzolar treatment was observed at either of the two incubation temperatures tested.

At both temperatures, flowers treated with the combined solutions of acibenzolar and MeJA (pulse or spray) showed no differences in disease reduction compared to those flowers treated with MeJA alone (pulse or spray) (Figure 4.17 A, B, C). Acibenzolar alone also provided protection at 12 and 20°C compared to untreated controls by slightly ($P < 0.05$) reducing disease severity, lesion numbers and lesion diameters (Figure 4.17; Appendix 4.4, Tables A4.4.4, A4.4.6 and A4.4.8). Lesion diameters on detached petals were significantly ($P < 0.05$) reduced compared to untreated controls by all treatments tested (Table 4.27; Appendix 4.4.1, Table A4.4.9).

Table 4.27: Lesion diameter on detached petals of freesia var 'Cote d'Azur' flowers gassed with 0.1 μL L^{-1} MeJA, pulsed with 200μM MeJA, sprayed with 600μM MeJA and with 0.15 g AI L^{-1} acibenzolar alone or in combined treatments. Flowers were then inoculated wit 10^4 *B. cinerea* conidial suspension and incubated at 12 and 20°C in the dark. Data are means of 10 replicate petals.

Treatments	**Lesion diameter (mm)** [a]
Control	4.8 c
MeJA gas (0.1 μL L^{-1})	3.4 a
MeJA pulse (200 μM)	3.7 ab
MeJA spray (600 μM)	3.6 ab
Acibenzolar (0.15 g AI L^{-1})	4.1 b
MeJA gas + Acibenzolar (0.1 μL L^{-1} + 0.15 g AI L^{-1})	3.6 ab
MeJA pulse + Acibenzolar (200 μM + 0.15 g AI L^{-1})	3.8 ab
MeJA spray + Acibenzolar (600 μM + 0.15 g AI L^{-1})	4.0 b

[a] Within detached petal variable, numbers followed by the same letter are not significantly different at P = 0.05.

4.1.4 Discussion

Gaseous MeJA at 0.1 μL L^{-1} and this MeJA treatment in combination with acibenzolar at 0.15 g AI L^{-1} provided the best suppression of *B. cinerea* specking on freesia flowers. Combined acibenzolar and MeJA applications did not provide synergistic protection against *B. cinerea* compared to MeJA or acibenzolar treatments alone. These findings agree with those in a previous study by Thomma *et al.* (2000). They showed in *Arabidopsis* plants treated with combined INA and MeJA, that there was no synergistic protection against *B. cinerea* disease. Disease suppression by MeJA gas was was greater at 20°C than at 12°C. Same MeJA reaction at high (20°C) and lower (12°C) incubation temperature was observed in experiment outlined in section 4.2.3.1.

4.5 EFFECTS OF POSTHARVEST UV-C IRRADIATION

4.5.1 Introduction

Low doses of ultraviolet light (UV) can reduce storage rots in fruits and vegetables (Stevens *et al.*, 1996). Disease reduction is attributed either to direct germicidal UV-C effects on the pathogen and/or to the defence response induction in the host following exposure to low UV-C doses (Chappell and Hahlbrock, 1984; Hahlbrock and Scheel, 1989; Stevens *et al.*, 1998; Marquenie *et al.*, 2001). *B. cinerea* conidial inactivation was progressively greater with increasing UV-C (0-20 kJ m^{-2}) doses and no conidia survived after 1 kJ m^{-2} UV-C exposure (Marquenie *et al.*, 2001). The efficacy of UV-C light against germination of *Monilinia fructicola* conidia increased linearly with UV-C doses from 0-20 kJ m^{-2} (Stevens *et al.*, 1998). Application of UV-C to harvested tomatoes at doses ranging from 1.3 to 43 kJ m^{-2} significantly reduced black mold (*A. alternata*), grey mold (*B. cinerea*) and Rhizopus soft rot (*R. stolonifer*) by defence response induction (Liu *et al.*, 1993). Nigro *et al.* (1998) showed that UV-C treatments of grapes at 0.125-4 kJ m^{-2} significantly reduced grey mould caused by *B. cinerea.*

The effects of UV-C irradiation on disease resistance activation in cut freesia flowers, and on *B. cinerea* conidial inactivation were investigated

4.5.2 Materials and methods

4.5.2.1 Plant material

Freesia flowers var 'Cote d' Azur' were provided by Zwetsloots & Sons Ltd (UK) (Appendix 2.2, Plate A2.1). Flowers were at the commercial harvest stage with all buds still closed (Appendix 2.2, Plate A2.2). They were processed in the laboratory approximately 24h after harvest.

4.5.2.2 UV-C treatment

Flowers were irradiated using three germicidal low-pressure vapour UV lamps (Osram HNS OFR). Each lamp (2.5 cm tube in diameter; 88cm length) had a nominal power output of 30W and peak wavelength emission of 253.7 nm (Terry, 2002). The lamps were assembled 15 cm apart and positioned 25 cm above the flowers (Nigro *et al.*, 1998). Irradiance was measured at 20°C using a Multi-Sense Optical Radiometer fitted with a 254 nm UV-C light sensor (Ultra-violet Products, Cambridge, UK). Flowers were arranged on plastic trays in a single layer and rotated on their longitudinal axis to expose the opposite sides of the cyme to the same UV-C dose (Nigro *et al.*, 1998).

Five UV-C doses of 0, 0.5, 1, 2.5, and 5 kJ m^{-2} were applied. Exposure time was calculated using the following equation:

Exposure time = Dose (D) / Dose rate (Dt) (Stevens *et al.*, 1999) (1).

Dose rate at 20°C was measured with the Multi-sense optical radiometer (MP-125UV-C sensor, Cambridge, UK) to be 0.64 mW cm^{-2}. Exposure times were calculated using equation (1) and determined to be: 0 kJ m^{-2} = 0 min., 0.5 kJ m^{-2} = 1.18 min., 1 kJ m^{-2} = 2.36 min., 2.5 kJ m^{-2} = 6.30 min. and 5 kJ m^{-2} = 13.00 min.

The irradiated flowers were inoculated with *B. cinerea* 24h after irradiation (Droby *et al.*, 1993; Nigro *et al.*, 1998). In order to assess any direct antifungal effect of UV-C on *B. cinerea*, another set of flowers were irradiated immediately after inoculation.

4.5.2.3 Inoculation and incubation

Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} or left non-inoculated as described in section 3.2.2.2. After inoculation, flowers were covered with plastic transparent bags (*ca.* 100% RH) and incubated at 20°C in the dark. All flowers were stood in individual 284 mL vases.

4.5.2.4 Disease assessments

Disease severity score, lesion numbers and lesion diameters on artificially inoculated freesia flowers were recorded using the scales described in section 3.2.2.4. Vase life, relative fresh weight and wilt score were also recorded using the scales described in section 4.1.2.5.

4.5.2.4 Experiment design and analysis

Flowers were arranged inside controlled temperature incubation rooms in a CRB design. Experimental factors were UV-C doses (0, 0.5, 1, 2.5 and 5 kJ m^{-2}) and inoculation time (before or after UV-C irradiation). Ten replicate flowers per treatment were used for disease assessments and 7 replicate flowers per treatment for vase life assessments. In the vase life experiment flowers were arranged inside a vase life room in a CR design. Data were analysed as described in section 4.1.2.8. Cubic regressions were used to describe the relationships between UV-C doses and disease parameters. Cubic regression analysis and graphic representation was performed using Sigmaplot 2000. Data in text are presented as main factor means in tables and the corresponding individual

treatment means are presented in figures. The results of the statistical tests are presented in appendices.

4.5.3 Results

4.5.3.1 Effect of UV-C irradiation on disease variables

Flower irradiation following artificial inoculation with *B. cinerea* generally conferred significant ($P < 0.05$) disease reduction compared to irradiation prior to artificial inoculation (Table 4.28; Appendix 4.5.1, Table A4.5.1.4, A4.5.1.6 and A4.5.1.8). Disease severity and lesion number on flowers irradiated after artificial inoculation were significantly ($P < 0.05$) lower compared to those irradiated prior to artificial inoculation at all UV-C doses tested (Table 4.28; Appendix 4.5.1, Table A4.5.1.4 and A4.5.1.6). Irradiation of artificially inoculated flowers with 0.5, 1, 2.5 or 5 kJ m^{-2} UV-C reduced disease severity and lesion numbers by up to 44, 70, 74 and 59% and by up to 37, 62, 68 and 60%, respectively (Figure 4.18).

In contrast, when flowers were irradiated prior to artificial inoculation no reduction in *B. cinerea* disease was observed. Lesion numbers on flowers irradiated with 0.5 or 1 kJ m^{-2} UV-C prior to artificial inoculation were slightly ($P < 0.05$) reduced compared to the un-irradiated control (Figure 4.18; Appendix 4.5.1, Table A4.5.1.6). Irradiation prior to artificial inoculation with the higher doses of 2.5 and 5 kJ m^{-2} resulted in greater disease pressure on irradiated flowers compared to non-irradiated controls. This was probably due to the UV injury effect.

Table 4.28: Effect of postharvest UV-C treatment on *B. cinerea* disease suppression on freesia var. 'Cote d'Azur' flowers. Flowers were treated with 0 (control), 0.5, 1, 2.5, or 5 kJ m^{-2} UV-C and inoculated with 10^4 *B. cinerea* conidia mL^{-1} either before or after UV-C irradiation. Disease assessments were carried out daily for 3 successive days after artificial inoculation. Data for independent treatment means are presented in Figure 4.18.

Factors	Disease variables		
	Disease severity (score 0-4) [a]	**Lesion number**	**Lesion diameter (mm)**
1) UV-C irradiation [b]			
Before inoculation	3.1 b	83 b	0.91 b
After inoculation	1.3 a	36 a	0.79 a
2) UV-C doses (kJ m^{-2})			
0	2.9 d	75 c	0.87 b
0.5	1.8 b	49 ab	0.79 a
1	1.5 a	43 a	0.80 a
2.5	1.9 b	52 b	0.85 ab
5	2.1 c	55 b	0.88 b

[a] Data are main factor means of disease severity, lesion number and lesion diameter.
[b] Within main factor means, numbers followed by the same letter are not significantly different at P = 0.05.

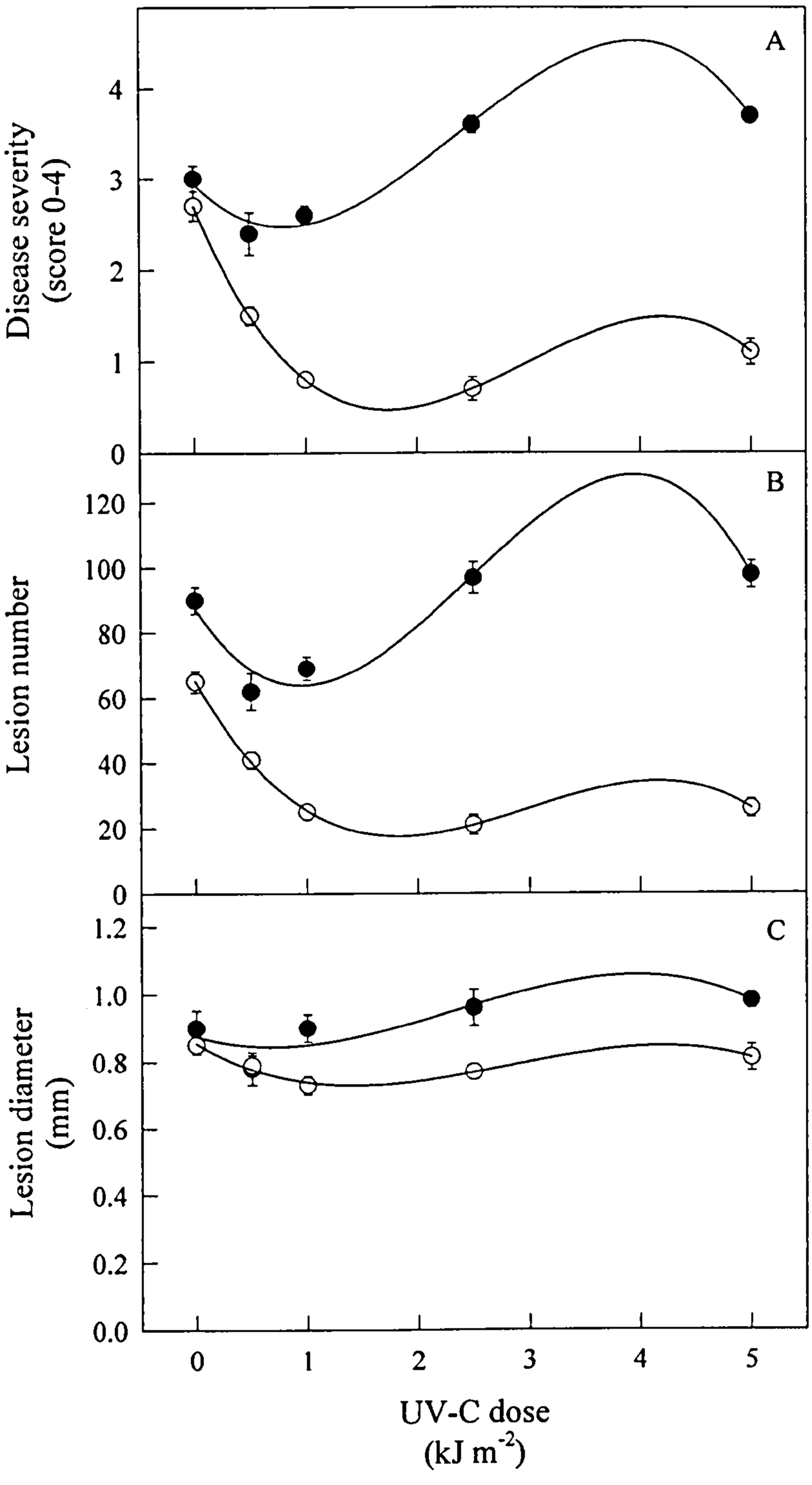

Figure 4.18: Effect of UV-C application time and doses on disease severity (A), lesion number (B) and lesion diameter (C). (●). UV-C treatment before inoculation, (○). UV-C treatment after inoculation. Vertical bars indicated the SE (n = 30). Data were collected over 3 successive days of incubation. Main factor means are presented in Table 4.28 and regression models are presented in Appendix 4.5.1, Table A4.5.1.9.

4.5.3.2 Effect of UV-C irradiation on freesia vase life

Flower fresh weight of UV-C irradiated flowers with 5 kJ m^{-2} was significantly ($P < 0.05$) less compared to un-irradiated control on days 2, 6 and 8 of incubation (Figure 4.19; Appendix 4.5.2, Tables A4.5.2.1 – A4.5.2.11).

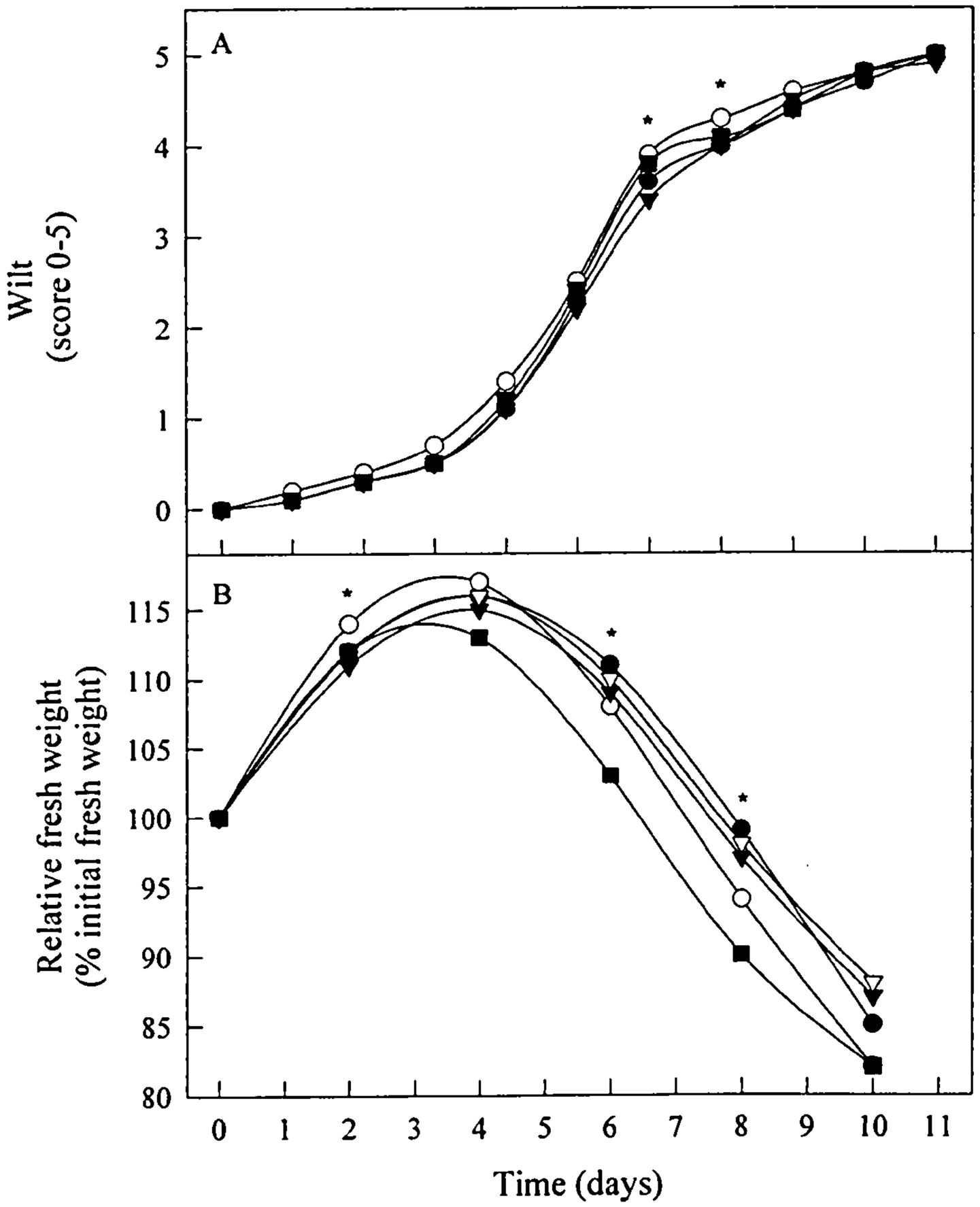

Figure 4.19: Wilt score (A) and relative fresh weight (B) of freesia flowers irradiated with 0 (●), 0.5 (○), 1 (▼), 2.5 (▽) or 5 (■) kJ m^{-2} UV-C and incubated at 20°C. Stars indicate significant difference between treatments at $P = 0.05$. Means were separated with Duncan's multiple range test at $P = 0.05$ ($n = 7$).

Vase life of flowers irradiated with 0.5, 1 or 2.5 kJ m^{-2} UV-C maintained at same levels with un-irradiated controls (Table 4.29; Appendix 4.5.2, Table A4.5.2.18). However, the vase life of flowers irradiated with 5 kJ m^{-2} UV-C was significantly ($P < 0.05$) reduced compared to control flowers and those exposed to low doses of 0.5 and 1 kJ m^{-2} (Table 4.29). Moreover, UV-C irradiation at 2.5 and 5 kJ m^{-2} caused phytotoxic symptoms on flower petals (Table 4.29). Phytotoxicity was visible as petal discoloration after 3 days of incubation.

Table 4.29: Vase life (days) of freesia var. 'Cote d'Azur' flowers irradiated with 0, 0.5, 1, 2.5 or 5 kJ m^{-2} UV-C and incubated at 20°C.

UV-C dose (kJ m^{-2})	Vase life (days) [a]	Phytotoxicity [b]
0	10.3 a	-
0.5	9.9 a	-
1	9.9 a	-
2.5	9.6 ab	+
5	8.9 b	+

[a] Within vase life variable, numbers followed by the same letter are not significantly different at $P = 0.05$.
[b] (+): presence, (-): absence of phytotoxicity symptoms.

4.5.4 Discussion

UV-C irradiation was effective in suppressing disease caused by *B. cinerea* when applied after artificial inoculation. UV-C irradiation seemed to have directly damaged *B. cinerea* conidia and reduced consequent infection of freesia petals. Marquenie *et al.* (2002) showed that UV-C irradiation at 1 kJ m^{-2} could inactivate *B. cinerea* conidia. Proportions of *Monilinia fructicola* conidial germination decreased with increasing UV-C doses from 0-20 kJ m^{-2} (Stevens *et al.*, 1998).

In the present study, attempts to induce disease resistance of cut freesia flowers with UV-C irradiation were successful only when low (eg. 0.5 and 1 kJ m^{-2}) UV-C doses were used. UV-C irradiation at 0.5 and 1 kJ m^{-2} before artificial inoculation slightly ($P <$

0.05) reduced *B. cinerea* disease severity and lesion numbers compared to un-irradiated controls. However, UV-C irradiation at 2.5 and 5 kJ m^{-2} facilitated *B. cinerea* infection after tissue damage due to high UV-C doses. Nigro *et al.* (1998), found that disease of table grapes infected by *B. cinerea* was reduced by up to 43% after UV-C irradiation, and this reduction was due to postharvest induction of PAL activity. Similarly, Liu *et al.* (1993) found that application of 1.3-40 kJ m^{-2} UV-C to harvested tomatoes markedly reduced black mold (*A. alternata*), grey mold (*B. cinerea*) and Rhizopus soft rot (*R. stolonifer*) by defense responses induction. Inhibition of *Monilinia fructicola, Alternaria* spp., *Colletotrichum gloeosporioides, Monilinia* spp., *P. digitatum, A. citri* and *Geotrichum candidum* via defence response induction after UV-C irradiation was reported for peaches, apples, mangoes, grapefruits and tangerines (Stevens *et al.*, 1996; Gonzalez-Aguilar *et al.*, 2001; El-Ghaouth *et al.*, 2003).

The present study suggests that UV-C irradiation might be used in an integrated postharvest disease management program for freesia flowers. Public concern over chemical use is increasing, and more producers are beginning to seek non-pesticide controls in order to better compete in the market. Postharvest UV-C irradiation could be a less expensive environmentally friendly method to reduce *B. cinerea* disease of freesia flowers. However, a number of limitations need to be addressed before UV-C application on freesia flowers is made practical (Wilson *et al.*, 1997). The effectiveness of UV-C light on postharvest disease can vary between commodities and across maturity stage (Droby *et al.*, 1993). Also, it is necessary to select the correct UV-C doses to optimise disease management without adversely affecting flower quality.

4.6 GENERAL CONCLUSIONS

Elicitation of cut flowers defence responses is an interesting prospect for *B. cinerea* disease control especially as it may offer alternatives to fungicide application. This series of postharvest experiments investigated the potential to induce natural defence mechanisms or directly controlling *B. cinerea* disease by application of biological and chemical elicitors. Some postharvest acibenzolar, MeJA and UV-C irradiation treatments markedly suppressed *B. cinerea* specking on freesia petals in terms of reducing disease

severity, lesion numbers and lesion diameters (Figure 4.20). However, elicitor efficacy varied with temperature and elicitor concentration. Briefly, MeJA treatment, independent of application mode, markedly reduced postharvest infection of freesia flowers at 20°C compared to untreated controls and rather than when incubation took place at 5°C. In contrast, acibenzolar was more effective at 5°C. Attempts to further minimise disease damage caused by *B. cinerea* using combined treatments at different plant activators, were not successful.

In summary, acibenzolar was not generally effective in reducing *B. cinerea* specking on cut freesia flowers (Figure 4.20). In addition, it remained unclear as to whether or not SAR was induced in cut freesia flowers after acibenzolar treatment. In contrast, gaseous MeJA treatment reduced disease severity most probably by inducing JA-dependant biochemical responses in freesia against *B. cinerea.* These contrasting results tend to concur with observations by Pieterse and van Loon (1999) and Thomma *et al.* (2001) that SA- versus JA-dependent pathways are effective against different pathogens. The results suggest that, SA-dependant pathway and consequently the SAR response was not effective in freesia flowers against *B. cinerea* infection. In contrast, the JA-dependant pathway was apparently induced and suppressive of *B. cinerea* infection.

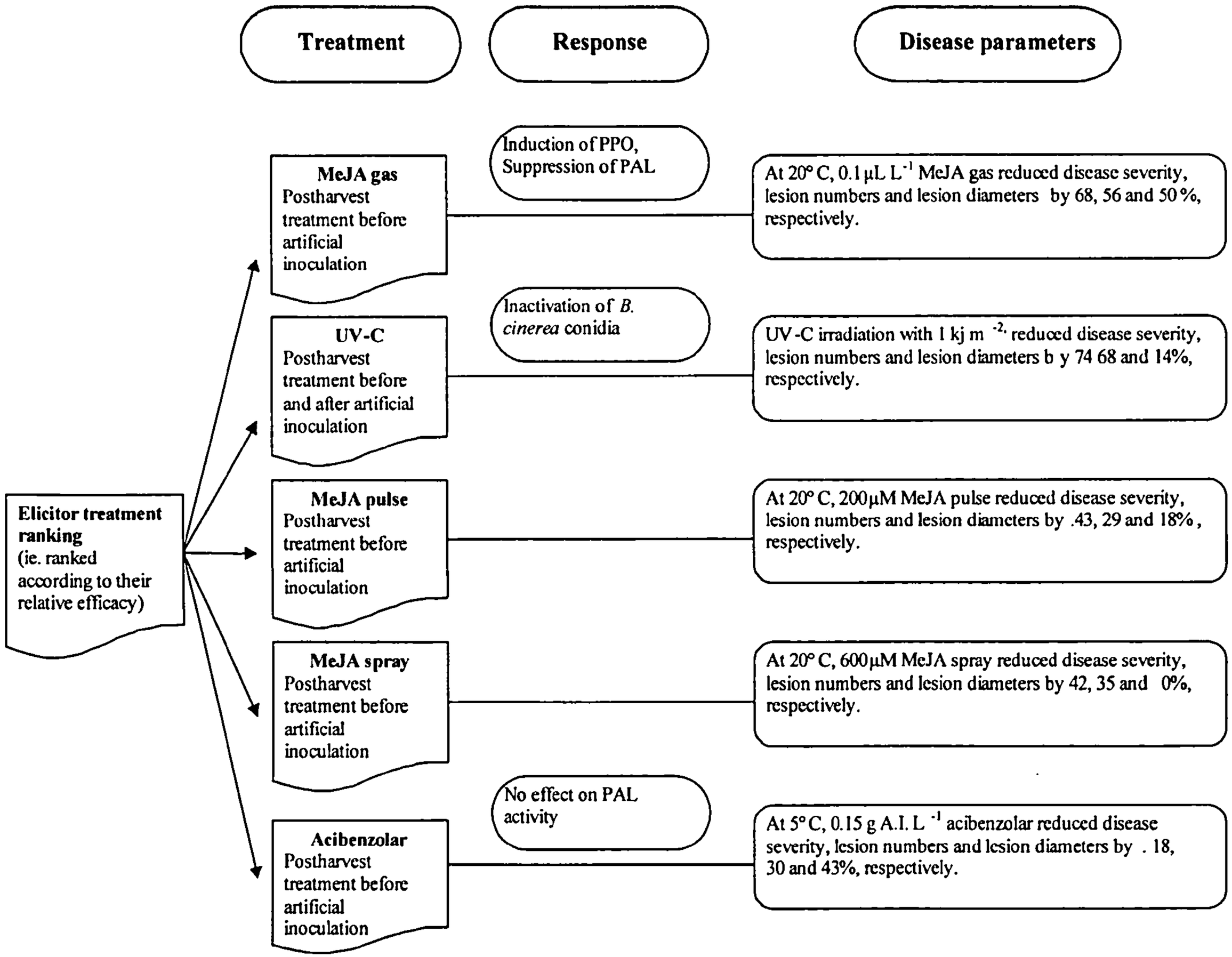

Figure 4.20: Ranking of postharvest elicitor treatments in terms of relative efficacy against *B. cinerea* starting with the most effective.

CHAPTER 5

GLASSHOUSE TRIALS OF ACIBENZOLAR-S-METHYL, *AUREOBASIDIUM PULLULLANS* AND METHYL JASMONATE TO SUPPRESS SPECKING ON CUT FREESIA FLOWERS CAUSED BY *BOTRYTIS CINEREA*

5.1 PREHARVEST ACIBENZOLAR-S-METHYL TREATMENTS

5.1.1 Introduction

Public concerns about synthetic chemical fungicide residues have caused major changes in pesticide use (Jacobsen and Backman, 1993). Novel strategies have been proposed to suppress development of various fungal pathogens on different crops as part of integrated pest management (IPM) systems (Agrios, 1997). These strategies include treatment with chemicals that activate plant immune reactions (Kessmann *et al.*, 1994). For example, acibenzolar-S-methyl a salicylic acid analogue, has been used on both dicots (e.g. soybean, Dann *et al.*, 1998) and monocots (e.g. wheat, Görlach *et al.*, 1996) to suppress *Sclerotinia sclerotiorum* and *Erisyphe graminis* f.sp. *tritici*, respectively (Table 2.5). Acibenzolar suppresses disease by inducing the systemic acquired resistance (SAR) (Kessmann *et al.*, 1994). Protein-encoding SAR-genes induce broad-spectrum resistance against pathogens during the onset of SAR (Kessmann *et al.*, 1994; Stermer, 1995; Ryals *et al.*, 1996; Sticher *et al.*, 1997).

The efficacy of preharvest treatments on freesia crops with acibenzolar was investigated in glasshouse trials to suppress postharvest *B. cinerea* infection during storage via possible SAR induction. Freesia flowers were treated with acibenzolar before harvest and artificially inoculated after harvest.

5.1.2 Materials and methods

5.1.2.1 Plant material, glasshouse and postharvest design

Freesia vars. 'Cinderella', 'Cote d'Azur' and 'Dukaat' were used. All corms were planted after chemical treatment with thiophanate-methyl (Topsin®, wetable powder 50%, Cercobin-M, USA) to prevent infection by *Fusarium* spp. Three glasshouse trials were run over 3 years. Corms were planted in early October 1999, 2000 and 2001 and flowers were harvested during April and May 2000, 2001 and 2002, respectively. Freesia plants were treated with acibenzolar and iprodione 28 days before harvest (Kessmann *et al.*, 1996). Disease parameters evaluated in harvested artificially inoculated flowers under storage conditions.

Acibenzolar-S-methyl glasshouse trial 2000

In the acibenzolar glasshouse trial in 2000, single freesia var. 'Cinderella' corms were planted into 9 cm diameter and 12 cm high pots (1 L capacity) on 8th of December 1999. The plants were grown inside an unheated glasshouse at Cranfield University at Silsoe (Bedfordshire, UK; 0° 25'W, 52° N). Fertigation was by watering with Tomorite Liquid Tomato Fertilizer (N-P-K: 4-4.5-8, Levington Ltd., England) at 20 mL 4.5 L^{-1} water. Fertilizer application commenced 5 weeks after planting and was applied once a week thereafter.

Freesia plants inside the glasshouse were arranged in CR design with chemical treatments as the single factor. There were eight chemical treatments of 0 (control), 0.15, 0.3 and 0.6 g AI L^{-1} acibenzolar, 0.5 g AI L^{-1} iprodione and all combinations of acibenzolar and iprodione (i.e. 0.15 g AI L^{-1} acibenzolar + 0.5 g AI L^{-1} iprodione, . 0.3 g AI L^{-1} acibenzolar + 0.5 g AI L^{-1} iprodione and 0.6 g AI L^{-1} acibenzolar + 0.5 g AI L^{-1} iprodione) Each treatment consisted of four replicate pots of one freesia plant per pot. After harvest, cut flowers were arranged inside controlled temperature incubation rooms in a CRB design. Postharvest experiments were two-factor designs with postharvest incubation temperatures (5, 12 and 20°C) and chemical treatments (0, 0.15, 0.3, 0.6 g AI

L-1 acibenzolar, 0.5 g AI L-1 iprodione, 0.15 g AI L-1 acibenzolar + 0.5 g AI L-1 iprodione, 0.3 g AI L-1 acibenzolar + 0.5 g AI L-1 iprodione and 0.6 g AI L-1 acibenzolar + 0.5 g AI L-1 iprodione) as factors.

Acibenzolar-S-methyl glasshouse trial 2001

In the acibenzolar glasshouse trial in 2001, freesia vars. 'Cinderella, 'Cote d'Azur and 'Dukaat' corms were planted in a non-heated commercial glasshouse at Zwetsloots & Sons Ltd. (Sandy, Bedfordshire, UK; 0° 18'W, 52° 07' N). Corms were planted in three beds each 33 m long x 1.12 m wide for 'Cinderella' and 'Dukaat' and 27 m long x 1.12 m wide for 'Cote d' Azur'. Soil was steam sterilized *in situ*. Corms were planted in early October 2001 and flowers were harvested during April and May 2002.

Freesia plots were arranged inside the glasshouse in a CR design in three separate variety beds. Within variety beds, five acibenzolar treatment plots of 0 (control), 0.15, 0.3, and 0.6 g AI L^{-1} acibenzolar, and 0.5 g AI L^{-1} iprodione were replicated three times. Thus, each acibenzolar treated bed was divided in 15 equal-sized plots. Ten replicate flowers were harvested for each variety. After harvest, cut flowers were arranged inside controlled temperature incubation rooms in a CRB design. Postharvest experiments were two-factor designs with postharvest incubation temperatures (5, 12 and 20°C) and chemical treatments (0, 0.15, 0.3, 0.6 g AI L^{-1} acibenzolar and 0.5 g AI L^{-1} iprodione) as factors.

Acibenzolar-S-methyl glasshouse trial 2002

In the acibenzolar glasshouse trial in 2002, vars 'Cinderella', 'Cote d'Azur' and 'Dukaat' corms were used. Soil was steam sterilised at Zwetsloots & Sons Ltd. and transported to Cranfield University at Silsoe by truck. Five freesia corms per variety were planted in 10 L capacity pots (26 x 18 x 26 cm) that were placed on benches in a non-heated glasshouse at Silsoe (Plate 5.1). Two heaters were used to prevent temperatures dropping below 0°C. Fertilization was not applied. Freesia plants were supported on string strung around four wooden sticks as shown on Plate 5.1.

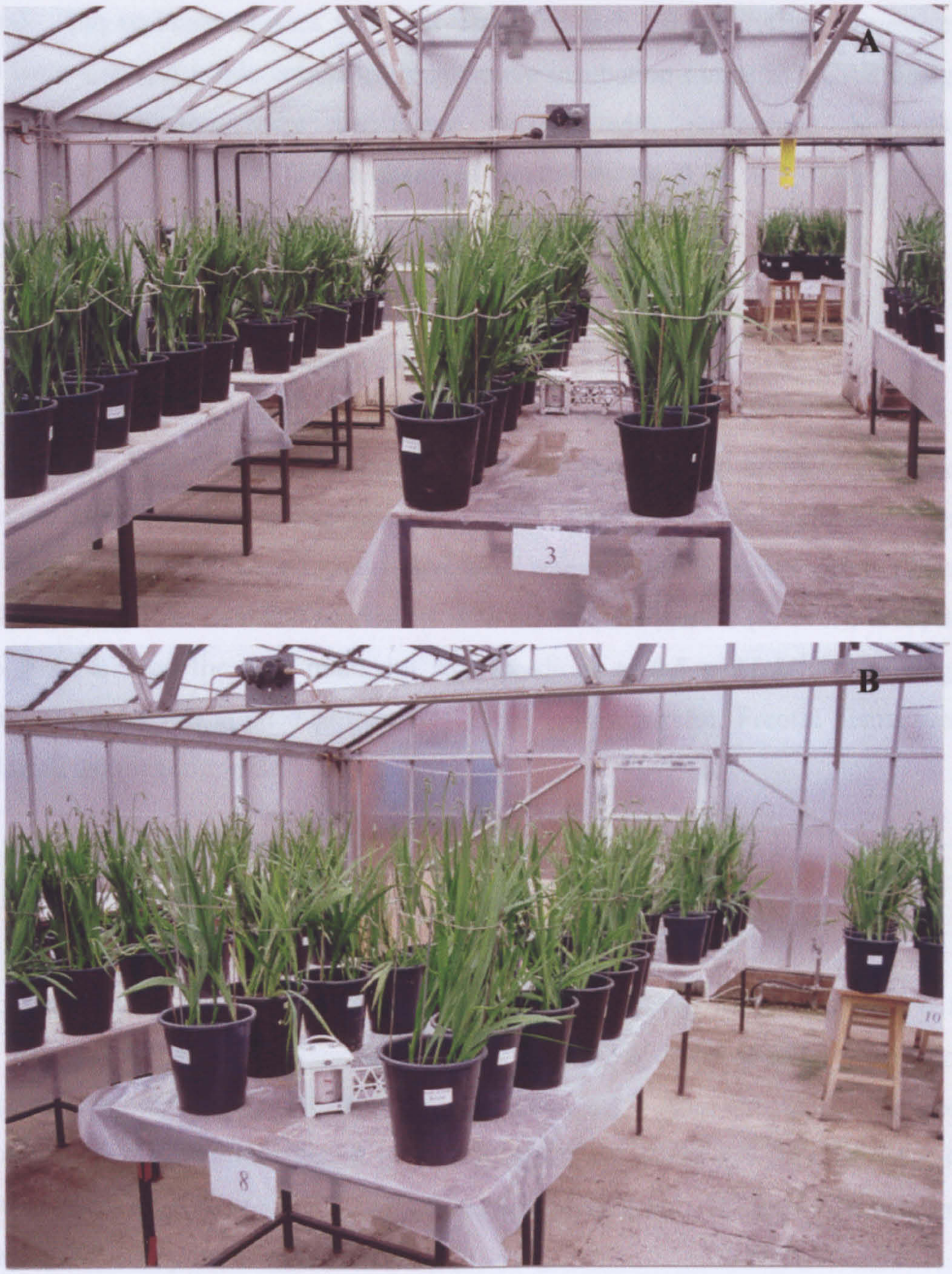

Plate 5.1: Acibenzolar glasshouse trial 2002. Freesia plants in pots arranged in a completely randomised block design on benches. Benches were arranged inside two glasshouse rooms. A: First glasshouse, and B: Second glasshouse room.

Pots inside the glasshouse were arranged on benches in a CRB design with chemical treatments and variety as the factors. There were 10 replication blocks

(benches). Ten replicate flowers were harvested for each variety. After harvest, cut flowers were arranged inside controlled temperature incubation rooms in a CRB design. Postharvest experiments were two-factor designs with postharvest incubation temperatures (5, 12 and 20°C) and chemical treatments (0, 0.15, 0,3, 0,6 g AI L^{-1} acibenzolar and 0.5 g AI L^{-1} iprodione) as factors.

5.1.2.2 Acibenzolar-S-methyl chemical treatment

Application of acibenzolar-S-methyl in the glasshouse trials in 2000, 2001, and 2002 started 28 days before harvest (Kessmann *et al.*, 1996). Thereafter, acibenzolar was applied every 7 days until harvest giving four serial sprays for each freesia variety (Ruess *et al.*, 1996). The four chemical treatments with acibenzolar were 0 (control), 0.15, 0.3 and 0.6 g AI L^{-1} and the fifth treatment was iprodione at 0.5 g AI L^{-1}. The single iprodione treatment was applied protectively 1 day before harvest. Freesia plants were sprayed until incipient run-off.

5.1.2.3 Postharvest flower inoculation and incubation

After harvest, flowers were taken to the laboratory and inoculated with a *B. cinerea* conidial suspension as described in section 3.2.2.2. The conidial suspension concentration was adjusted to 10^4 *B. cinerea* conidia mL^{-1}. After inoculation, flowers were covered with plastic transparent bags to maintain *ca.* 100% RH (section 4.1.2.3). They were then placed at 5, 12 and 20°C for incubation. Disease assessments commenced 24h after inoculation.

5.1.2.4 Disease assessments

Botrytis disease severity, lesion numbers, lesion diameters and senescence were recorded postharvest using the scales described in section 3.2.2.4 (Appendix 2.2, Plate

A2.3). Lesion numbers on petals were counted every day after artificial inoculation. Diameters of 10 randomly selected lesions per flower were measured each day as described in section 3.2.2.4.

5.1.2.5 Phenylalanine ammonia lyase (PAL) analysis

In the glasshouse trial in 2001, PAL activity was measured in selected treatments as a possible index of elicited secondary metabolite synthesis after acibenzolar treatment. PAL activity was measured as described in section 5.1.2.5.

5.2.2.6 Statistical analysis

Data were analysed as described in section 4.2.1.8. Data in text are presented as individual treatment means in tables.

5.1.3 Results

5.1.3.1 Effect of preharvest acibenzolar-S-methyl treatment (glasshouse trial 2000)

Acibenzolar treatment on freesia var. 'Cinderella' flowers before harvest, markedly ($P < 0.05$) reduced disease severity compared to untreated controls (Table 5.1; Appendix 5.1.1, Table A5.1.1.3). Iprodione treatments also significantly ($P < 0.05$) reduced *B. cinerea* disease severity compared to untreated controls (Table 5.1; Appendix 5.1.1, Table A5.1.1.3).

No additional disease reduction was observed for combined acibenzolar and iprodione treatments. However, greater disease severity reduction was observed on freesia flowers treated with 0.15 g AI L^{-1} acibenzolar plus 0.5 g AI L^{-1} iprodione compared to untreated controls.

Table 5.1: Effects of preharvest acibenzolar-S-methyl and iprodione treatments alone or in combination on *B. cinerea* disease in freesia var. 'Cinderella' flowers from a glasshouse trial in 2000. Flowers were artificially inoculated postharvest with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C in the dark. Disease assessments were carried out daily for 4 successive days of incubation.

Treatments	**Disease severity** [a]
Control (untreated)	3.6 c
Acibenzolar (0.15 g AI L^{-1})	2.5 b
Acibenzolar (0.3 g AI L^{-1})	1.2 a
Acibenzolar (0.6 g AI L^{-1})	1.6 ab
Iprodione (0.5 g AI L^{-1})	2.0 ab
Acibenzolar (0.15 g AI L^{-1}) + (0.5 g AI L^{-1}) Iprodione	1.1 a
Acibenzolar (0.3 g AI L^{-1}) + (0.5 g AI L^{-1}) Iprodione	1.4 a
Acibenzolar (0.6 g AI L^{-1}) + (0.5 g AI L^{-1}) Iprodione	1.8 ab

[a] Numbers followed by the same letter are not significantly different at P = 0.05.

5.1.3.2 Effect of preharvest acibenzolar-S-methyl treatment (glasshouse trial 2001)

Incubation temperature and chemical treatments had slight ($P < 0.05$) effects on disease severity, lesion numbers and lesion diameters in all three varieties tested (Tables 5.2, 5.3, and 5.4). Acibenzolar provided greater protection in reducing *B. cinerea* disease severity, lesion numbers and lesion diameters on freesia var. 'Cote d'Azur' flowers (Table 5.3; Appendix 4.1.2, Tables A5.1.2.12, A5.1.2.14 and A5.1.2.16). Limited protection was observed in acibenzolar treated 'Cinderella' and 'Dukaat' flowers (Tables 5.2 and 5.4). Disease severity, lesion number and lesion diameter reduction did not significantly differ within the acibenzolar concentrations of 0.15, 0.3 and 0.6 g AI L^{-1}. However, lesion diameter was significantly ($P < 0.05$) reduced following acibenzolar treatment in all three varieties (Tables 5.2, 5.3, and 5.4). Iprodione treatment markedly ($P < 0.05$) reduced disease severity, lesion numbers and lesion diameters in 'Cinderella' flowers (Table 5.2; Appendix 4.1.2, Tables A5.1.2.4, A5.1.2.6 and A5.1.2.8). The fungicide also reduced disease severity and lesion number in 'Cote d'Azur' (Table 5.3; Appendix 4.1.2, Tables A5.1.2.12, A5.1.2.14 and A5.1.2.16) and 'Dukaat' flowers (Tables 5.3; Appendix 4.1.2, Tables A5.1.2.20, A5.1.2.22 and A5.1.2.24). Independent

of acibenzolar or iprodione treatment and variety tested, flowers at 5°C showed significantly ($P < 0.05$) lower disease severity, lesion number and lesion diameter main factor means compared to flowers incubated at 12 and 20°C (Tables 5.2, 5.3, and 5.4).

Table 5.2: Effects of preharvest treatments with acibenzolar-S-methyl and iprodione on *B. cinerea* disease for freesia var. 'Cinderella' flowers from the glasshouse trial in 2001. Flowers were sprayed with 0 (control), 0.15, 0.30, and 0.60 g AI L^{-1} acibenzolar and 0.50 g AI L^{-1} iprodione. They were artificially inoculated postharvest with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C in the dark. Disease assessments were carried out daily over 4 successive days of incubation.

Acibenzolar-S-methyl concentration (g AI L^{-1})	**Temperature (°C) [a]**			**Row means [b]**
	5	**12**	**20**	
No. of flower samples	10	10	10	120
	a. Disease severity (score 0-4)			
0 (control)	1.8 de	1.9 de	1.7 cde	1.8 C
0.15	1.2 ab	1.5 bcd	1.9 de	1.5 AB
0.30	1.0 ab	1.8 de	1.9 de	1.6 BC
0.60	1.5 bcd	1.7 cd	2.2 e	1.8 C
Iprodione	0.9 a	1.7 cd	1.3 abc	1.3 A
Column means (n = 200) [c]	1.3 A	1.7 B	1.8 B	
	b. Lesion number			
0 (control)	42 bc	63 d	62 d	56 C
0.15	29 ab	54 cd	61 d	48 BC
0.30	24 a	53 cd	56 cd	45 AB
0.60	47 cd	59 d	61 d	56 C
Iprodione	23 a	51 cd	41 bc	38 A
Column means (n = 200)	33 A	56 B	56 B	
	c. Lesion diameter (mm)			
0 (control)	0.8 ab	1.1 c	1.0 c	1.0 C
0.15	0.8 ab	0.7 ab	1.1 c	0.9 BC
0.30	0.7 ab	0.8 ab	0.9 b	0.8 AB
0.60	0.7 ab	0.7 ab	0.9 b	0.8 AB
Iprodione	0.6 a	0.7 ab	0.8 ab	0.7 A
Column means (n = 200)	0.7 A	0.8 A	0.9 B	

[a] For the acibenzolar treatment by temperature interaction, numbers followed by the same letter are not significantly different at P = 0.0.5. [b] Within row means numbers followed by the same letter are not significantly different at P = 0.0.5. [c] Within column means numbers followed by the same letter are not significantly different at P = 0.0.5.

Table 5.3: Effects of preharvest treatments with acibenzolar-S-methyl and iprodione on *B. cinerea* disease for freesia var. 'Cote d'Azur' flowers from the glasshouse trial in 2001. Flowers were sprayed with 0 (control), 0.15, 0.30, and 0.60 g AI L^{-1} acibenzolar and 0.50 g AI L^{-1} iprodione. They were artificially inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C in the dark. Disease assessments were carried out daily over 4 successive days of incubation.

Acibenzolar-S-methyl concentration (g AI L^{-1})	**Temperature (°C) [a]**			**Row means [b]**
	5	**12**	**20**	
No. of flower samples	8	8	8	96
	a. Disease severity (score 0-4)			
0 (control)	3.1 cde	3.5 g	3.5 fg	3.3 B
0.15	1.9 a	2.8 bcde	3.1 efg	2.6 A
0.30	1.6 a	2.7 bc	2.9 cde	2.4 A
0.60	1.8 a	2.7 bcd	3.1 def	2.5 A
Iprodione	1.8 a	3.0 cde	2.5 b	2.4 A
Column means (n = 160) [c]	2.1 A	2.9 B	3.0 B	
	b. Lesion number			
0 (control)	87 gh	122 i	86 gh	100 C
0.15	54 ab	81 efgh	71 cdef	70 B
0.30	48 a	73 defg	60 abcd	60 A
0.60	67 bcde	85 fgh	56 ab	69 B
Iprodione	52 a	91 h	58 abc	68 AB
Column means (n = 160)	62 A	91 B	66 A	
	c. Lesion diameter (mm)			
0 (control)	0.9 bcd	1.1 f	1.0 de	1.0 B
0.15	0.7 a	0.9 bcd	0.8 abcd	0.8 A
0.30	0.7 a	0.9 bcd	0.8 ab	0.8 A
0.60	0.8 abc	0.9 cd	0.9 bcd	0.8 A
Iprodione	0.9 bcd	1.1 ef	0.9 abcd	0.9 B
Column means (n = 160)	0.8 A	1.0 C	0.9 B	

[a] For the acibenzolar treatment by temperature interaction, numbers followed by the same letter are not significantly different at P = 0.0.5. [b] Within row means numbers followed by the same letter are not significantly different at P = 0.0.5. [c] Within column means numbers followed by the same letter are not significantly different at P = 0.0.5.

Table 5.4: Effects of preharvest treatments with acibenzolar-S-methyl and iprodione on *B. cinerea* disease for freesia var. 'Dukaat' flowers from the glasshouse trial in 2001. Flowers were sprayed with 0 (control), 0.15, 0.30, and 0.60 g AI L^{-1} acibenzolar and 0.50 g AI L^{-1} iprodione. They were artificially inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C in the dark. Disease assessments were carried out daily over 4 successive days of incubation.

Acibenzolar-S-methyl concentration (g AIL^{-1})	**Temperature (°C) [a]**			**Row means [b]**
	5	**12**	**20**	
No. of flower samples	10	10	10	120
	a. Disease severity (score 0-4)			
0 (control)	1.0 bcd	1.5 e	1.0 abc	1.2 B
0.15	0.8 ab	1.2 cde	1.1 bcd	1.0 B
0.30	1.1 bcd	1.3 de	0.9 abc	1.1 B
0.60	1.0 bcd	1.5 e	1.1 bcd	1.2 B
Iprodione	0.6 a	0.9 abc	0.8 ab	0.8 A
Column means (n = 200) [c]	0.9 A	1.3 B	1.0 B	
	b. Lesion number			
0 (control)	66 ef	95 h	62 de	75 D
0.15	45 abc	67 ef	46 abc	53 AB
0.30	58 bcde	80 fg	49 abcd	64 C
0.60	48 abcd	82 gh	45 ab	60 BC
Iprodione	36 a	61 cde	54 bcde	50 A
Column means (n = 200)	51 A	77 B	51 A	
	c. Lesion diameter (mm)			
0 (control)	0.6 cde	0.7 f	0.7 ef	0.7 B
0.15	0.6 abcd	0.5 a	0.6 abcd	0.5 A
0.30	0.6 cde	0.5 abcd	0.6 abcd	0.5 A
0.60	0.5 ab	0.5 abc	0.6 de	0.5 A
Iprodione	0.6 cde	0.5 abcd	0.6 bcde	0.6 A
Column means (n = 200)	0.6 AB	0.5 A	0.6 B	

[a] For the acibenzolar treatment by temperature interaction, numbers followed by the same letter are not significantly different at P = 0.0.5. [b] Within row means numbers followed by the same letter are not significantly different at P = 0.0.5. [c] Within column means numbers followed by the same letter are not significantly different at P = 0.0.5.

5.1.3.3 Effect of preharvest acibenzolar-S-methyl treatment (glasshouse trial 2002)

The effect of acibenzolar on *B. cinerea* postharvest disease was incosistent for the glasshouse trials in both 2001 and 2002. In glasshouse trial in 2001 disease levels in all three varieties independent of chemical treatment, were markedly higher compared to disease levels recorded in glasshouse trial in 2002. Temperature and chemical treatments significantly ($P < 0.05$) affected disease severity, lesion numbers and lesion diameters (Tables 5.5, 5.6, and 5.7) in 2002. Acibenzolar provided limited protection for all three varieties. As found in 2001, acibenzolar was again better in reducing disease severity, lesion numbers and lesion diameters on freesia var. 'Cote d'Azur' (Table 5.6; Appendix 5.1.3, Table A5.1.3.4, A5.1.3.6 and A5.1.3.8) and 'Dukaat' (Table 5.7; Appendix 5.1.3, Tables A5.1.3.20, A5.1.3.22 and A4.1.3.24) flowers compared to untreated controls. However, disease severity, lesion numbers and lesion diameters on acibenzolar treated 'Cinderella' freesia flowers were not, in most cases, significantly ($P > 0.05$) reduced compared to untreated controls at 5, 12 or 20 (Table 5.5; Appendix 5.1.3, Tables A5.1.3.12, A5.1.3.14 and A5.1.3.16). In the 2002 acibenzolar glasshouse trial, in contrast to that in 2001, disease severity, lesion numbers and lesion diameters were reduced in more cases in acibenzolar treated flowers incubated at 20°C compared to untreated controls in all three varieties tested (Tables 5.5, 5.6, and 5.7).

Iprodione did not provide the same high degree of protection compared to that observed in the 2001 glasshouse trial (Tables 5.5, 5.6, and 5.7). Iprodione was slightly ($P < 0.05$) effective in reducing disease severity compared to untreated controls on 'Cinderella' and Cote d'Azur' flowers incubated at 12 and 20°C, respectively (Tables 5.5 and 5.6). Also iprodione was effective ($P < 0.05$) in reducing lesion numbers on 'Dukaat' flowers incubated at 12°C compared to untreated controls.

Table 5.5: Effects of preharvest treatments with acibenzolar-S-methyl and iprodione on *B. cinerea* disease for freesia var. 'Cinderella' flowers from the glasshouse trial in 2002. Flowers were sprayed with 0 (control), 0.15, 0.30, and 0.60 g AI L^{-1} acibenzolar and 0.50 g AI L^{-1} iprodione. They were artificially inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C in the dark. Disease assessments were carried out daily over 3 successive days of incubation.

Acibenzolar-S-methyl concentration (g AI L^{-1})	**Temperature (°C) [a]**			**Row means [b]**
	5	**12**	**20**	
No. of flower samples	10	10	10	90
	a. Disease severity (score 0-4)			
0 (control)	0.0 a	0.8 e	0.9 e	0.6 B
0.15	0.1 a	0.8 cde	0.5 bc	0.4 AB
0.30	0.2 ab	0.8 de	0.6 cde	0.5 AB
0.60	0.2 ab	0.8 cde	0.5 cde	0.5 AB
Iprodione	0.1 a	0.6 cde	0.4 bc	0.4 A
Column means (n = 150) [c]	0.1 A	0.8 C	0.6 B	
	b. Lesion number			
0 (control)	2 a	27 cde	40 e	23 A
0.15	4 a	25 cde	17 abc	16 A
0.30	10 ab	33 de	28 cde	23 A
0.60	7 ab	21 bcd	21 bcd	16 A
Iprodione	3 a	30 cde	27 cde	16 A
Column means (n = 150)	5 A	27 B	27 B	
	c. Lesion diameter (mm)			
0 (control)	0.5 ab	0.6 bcdef	0.7 g	0.6 B
0.15	0.6 bcd	0.7 efg	0.6 bcd	0.6 B
0.30	0.6 bcd	0.6 bcdef	0.5 a	0.6 A
0.60	0.6 defg	0.6 bcd	0.6 cdefg	0.6 B
Iprodione	0.5 abc	0.6 cdefg	0.7 fg	0.6 B
Column means (n = 150)	0.6 A	0.6 A	0.6 A	

[a] For the acibenzolar treatment by temperature interaction, numbers followed by the same letter are not significantly different at P = 0.0.5. [b] Within row means numbers followed by the same letter are not significantly different at P = 0.0.5. [c] Within column means numbers followed by the same letter are not significantly different at P = 0.0.5.

Table 5.6: Effects of preharvest treatments with acibenzolar-S-methyl and iprodione on *B. cinerea* disease for freesia var. 'Cote d'Azur' flowers from the glasshouse trial in 2002. Flowers were sprayed with 0 (control), 0.15, 0.30, and 0.60 g AI L^{-1} acibenzolar and 0.50 g AI L^{-1} iprodione. They were artificially inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C in the dark. Disease assessments were carried out daily over 2 days of incubation.

Acibenzolar-S-methyl concentration (g AI L^{-1})	**Temperature (°C) [a]**			**Row means [b]**
	5	**12**	**20**	
No. of flower samples	20	20	20	60
	a. Disease severity (score 0-4)			
0 (control)	0.6 a	2.6 de	2.8 e	2.0 B
0.15	0.5 a	2.3 cde	1.7 b	1.5 A
0.30	0.5 a	2.3 cde	2.1 bc	1.6 A
0.60	0.7 a	2.3 cde	2.1 bcd	1.7 A
Iprodione	0.6 a	2.0 bc	2.7 e	1.8 AB
Column means (n = 100) [c]	0.6 A	2.3 B	2.3 B	
	b. Lesion number			
0 (control)	14 a	77 cd	95 e	62 C
0.15	11 a	71 c	57 b	46 A
0.30	15 a	69 bc	74 c	52 AB
0.60	14 a	76 cd	76 cd	55 CB
Iprodione	13 a	65 bc	89 de	56 CB
Column means (n = 100)	14 A	72 B	78 C	
	c. Lesion diameter (mm)			
0 (control)	0.8 ab	0.9 bc	1.0 bc	0.9 B
0.15	0.9 ab	0.8 a	0.8 a	0.8 A
0.30	0.8 ab	0.8 ab	0.7 a	0.8 A
0.60	0.8 ab	0.7 a	0.8 ab	0.8 A
Iprodione	1.1 c	1.0 c	1.0 c	1.0 C
Column means (n = 100)	0.9 A	0.9 A	0.9A	

[a] For the acibenzolar treatment by temperature interaction, numbers followed by the same letter are not significantly different at P = 0.0.5. [b] Within row means numbers followed by the same letter are not significantly different at P = 0.0.5. [c] Within column means numbers followed by the same letter are not significantly different at P = 0.0.5.

Table 5.7: Effects of preharvest treatment with acibenzolar-S-methyl and iprodione on *B. cinerea* disease for freesia var. 'Dukaat' flowers from the glasshouse trial in 2002. Flowers were sprayed with 0 (control), 0.15, 0.30, and 0.60 g AI L^{-1} acibenzolar and 0.50 g AI L^{-1} iprodione. They were artificially inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C in the dark. Disease assessments were carried out daily over 3 days of incubation.

Acibenzolar-S-methyl concentration (g AI L^{-1})	**Temperature (°C) [a]**			**Row means [b]**
	5	**12**	**20**	
No. of flower samples	10	10	10	90
	a. Disease severity (score 0-4)			
0 (control)	0.7 ab	1.6 f	1.1 cde	1.1 D
0.15	0.4 a	1.2 def	0.5 a	0.7 A
0.30	0.4 a	1.4 ef	0.5 a	0.8 AB
0.60	0.5 a	1.4 ef	0.9 bc	0.9 BC
Iprodione	0.6 a	1.5 f	1.0 bcd	1.0 CD
Column means (n = 150) [c]	0.5 A	1.4 C	0.8 B	
	b. Lesion number			
0 (control)	25 abc	65 e	35 c	42 C
0.15	14 a	48 d	17 a	26 A
0.30	16 a	54 de	18 a	29 A
0.60	20 ab	47 d	31 bc	33 AB
Iprodione	20 ab	31 bc	31 bc	37 BC
Column means (n = 150)	19 A	55 C	26 B	
	c. Lesion diameter (mm)			
0 (control)	0.7 bcde	0.7 bcde	0.7 bcde	0.7 B
0.15	0.7 bcde	0.6 ab	0.5 a	0.6 A
0.30	0.7 bcde	0.7 cde	0.7 e	0.7 B
0.60	0.6 bc	0.7 cde	0.7 cde	0.7 B
Iprodione	0.6 bc	0.7 bcde	0.7 de	0.7 B
Column means (n = 150)	0.6 A	0.7 A	0.7 A	

[a] For the acibenzolar treatment by temperature interaction, numbers followed by the same letter are not significantly different at P = 0.0.5. [b] Within row means numbers followed by the same letter are not significantly different at P = 0.0.5. [c] Within column means numbers followed by the same letter are not significantly different at P = 0.0.5.

5.1.3.4 PAL activity in acibenzolar treated flowers

There were few significant ($P > 0.05$) differences in PAL activity of 'Dukaat' flowers among acibenzolar and untreated controls at either of the two incubation temperatures (Figure 5.1 A and B; Appendix 5.1.4, Tables A5.1.4.1–A5.1.4.8).

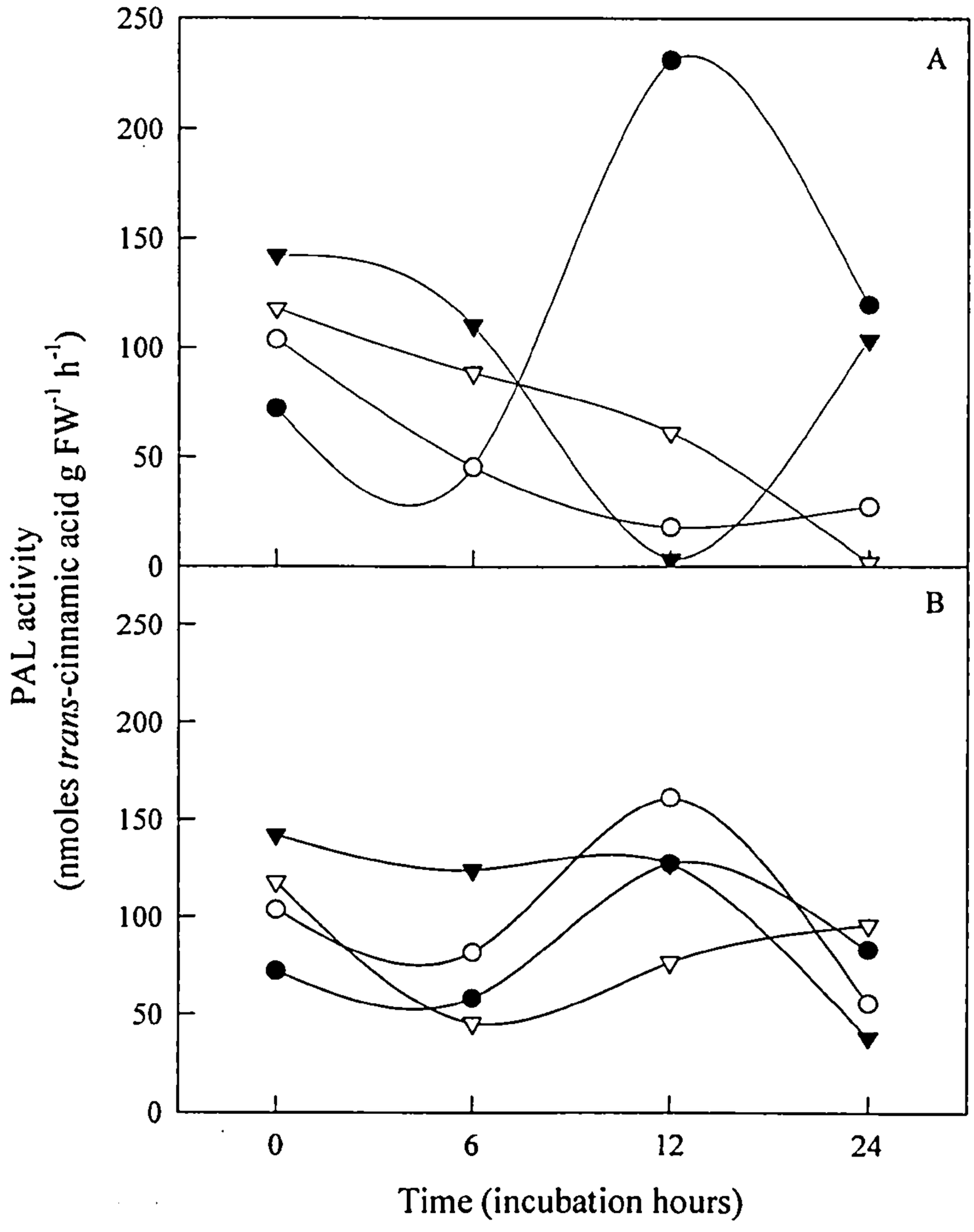

Figure 5.1: PAL activity in freesia var. 'Dukaat' flower petals treated with 0 (control) (●), 0.15 (○), 0.3 (▽), and 0.6 (▼) g AI L^{-1} acibenzolar and incubated at 12°C (A) and 20°C (B) for 24h. Data are means of three replicates each consisted of 3 flowers. $SEM_{12°C}$ = 8.46, $SEM_{20°C}$ = 7.57 (n = 3).

At 12°C, flowers treated at all three acibenzolar rates, had similar PAL levels. However, untreated controls showed markedly ($P < 0.05$) higher PAL activity than all acibenzolar concentrations after 12h of incubation. PAL activities after 24h of incubation for control flowers were also significantly ($P < 0.05$) higher than those treated with 0.15 and 0.6 g AI L^{-1} (Figure 5.1 A; Appendix 5.1.4, Tables A5.1.4.1–A5.1.4.4). Finally, PAL activity in acibenzolar treated 'Dukaat' flowers at 0.15 and 0.6 g AI L^{-1} followed a gradual decline over the 24h period of incubation at 12°C.

At 20°C, PAL activity of acibenzolar treated flowers at 0 (control), 0.15. 0.3 and 0.6 g AI L^{-1} followed similar patterns of change (Figure 5.1 B; Appendix 5.1.4, Tables A5.1.4.5–A5.1.4.8). PAL activities did not significantly ($P > 0.05$) change throughout the 24h of incubation. After 6 hours incubation, PAL activity of acibenzolar treated with 0.3 g AI L^{-1} flowers was significantly ($P < 0.05$) higher than in those treated with 0.6 g AI L^{-1} (Figure 5.1 B; Appendix 5.1.4, Tables A5.1.4.5–A5.1.4.8). The opposite result of difference between 0.3 g AI L^{-1} and 0.6 g AI L^{-1} was recorded 24h after inoculation (Figure 5.1 B; Appendix 5.1.4, Tables A5.1.4.5–A5.1.4.8).

5.1.4 Discussion

Acibenzolar-S-methyl slightly reduced *B. cinerea* disease severity on 'Cinderella', 'Cote d'Azur' and 'Dukaat' freesia flowers. Lesion numbers and size were generally reduced at all incubation temperatures, although not consistently over all concentrations, incubation temperatures and varieties. Inconsistent effects of acibenzolar in terms of failure to induce systemic protection in some crops against various diseases have been reported (Bokshi *et al.*, 2000; van Toor *et al.*, 2001). For example, infection of camellia (*Camellia japonica*) flowers by *Ciborinia camelliae* in field trials in New Zealand was not reduced by acibenzolar treatment (van Toor *et al.*, 2001).

Iprodione provided inconsistent levels of protection of cut freesia flowers by *B. cinerea*. Previous studies on other ornamentals have demonstrated the high efficacy of iprodione (Elad, 1988; Elad *et al.*, 1993a; Elad *et al.*, 1993b; Taylor *et al.*, 1999). Positive and consistent iprodione effects were observed during the glasshouse trials in

2000 and 2001. However, iprodione provided only limited protection in glasshouse trial in 2002.

In the 2001 and 2002 trials, protection by acibenzolar was variety dependent. Acibenzolar treatments markedly protected var. 'Cote d'Azur' flowers than 'Cinderella and 'Dukaat' flowers. 'Cote d'Azur' was the most susceptible variety in these glasshouse trials as shown by disease levels in untreated controls. Moreover, 'Dukaat' was the most resistant. Similar findings of acibenzolar efficacy over resistant or susceptible varieties were reported by Dann *et al.* (1998) on soybean during field and glasshouse trials. It may be that enhancement of physiological defence responses by chemical activators such as acibenzolar on varieties that already have a high degree of resistance is not as potent as on varieties that have a lower degree of resistance (Dann *et al.*, 1998).

In the 2001 acibenzolar glasshouse trial, PAL activity in acibenzolar treated flowers was no higher than in untreated control flowers. Furthermore, PAL levels in untreated controls were higher than in acibenzolar treated flowers at 12°C after 12 and 24h of incubation. Thus, acibenzolar treatment apparently did not induce the anticipated biochemical change of increased PAL activity (Kuć, 1995; Kombrink and Somssich, 1995).

5.2 *AUREOBASIDIUM PULLULLANS* PREHARVEST TREATMENTS

5.2.1 Introduction

Utilisation of antagonistic yeasts as alternatives to synthetic fungicides appears to be a promising strategy in achieving IPM (Castoria *et al.*, 2001). The yeast-like fungus *A. pullulans* was an effective antagonist against *B. cinerea* and *Rhizopus stolonifer* on strawberry fruits (Lima *et al.*, 1997). Pre-harvest treatment with *A. pullulans* has shown significant antagonistic activity against *B. cinerea,* on table grapes, and on cherry tomato (Schena *et al.*, 1999; Castoria *et al.*, 2001). In biochemical analysis, extracellular exo-chitinase [N-acetyl-β-D-glucosaminidase (Nagase)] and β-1,3-glucanase activities were detected after *A. pullulans* treatment both *in-vitro* and in apple wounds, which were the

main penetration sites of postharvest fungal pathogens (Castoria *et al.*, 2001). According to Adikaram *et al.* (2002), control of grey mould on green strawberry fruits was a result of induced preformed compounds and phytoalexins as shown by thin layer chromatography bioassays. Also, Ippolito *et al.* (2000a) showed that *A. pullulans* postharvest treatment on apple fruits markedly induced chitinase, β-1,3 glucanase and peroxidase activity 48h after treatment.

The aim of the present study was to examine the effect of *A. pullulans* applied before harvest to suppress postharvest infection by *B. cinerea*. In this study, we evaluated both postharvest antagonistic and induced activity following *A. pullulans* preharvest treatment.

5.2.2 Materials and methods

5.2.2.1 Plant material, glasshouse and postharvest designs and statistical analysis

A glasshouse trial testing *A. pullulans* was carried out in 2001. Freesia vars. 'Cinderella, 'Cote d'Azur and 'Dukaat' were planted in a non-heated glasshouse at Zwetsloots & Sons Ltd. (Sandy, UK; 0° 18'W, 52° 07' N). The corms were planted in three freesia-beds that were 33 m long x 1.12 m wide for 'Cinderella' and 'Dukaat' and 27 m long x 1.12 m wide for 'Cote d' Azur'. Soil was steam sterilized *in-situ*. Corms were planted in early October 2001 and flowers were harvested during April and May 2002. The three parallel experiments were conducted in each of the three separate freesia-variety beds. Within each variety beds two *A. pullulans* treatment plots were replicated three times. Thus, cach *A. pullulans* treated bed was divided in 6 equal-sized plots. After harvest, cut flowers were arranged inside controlled temperature incubation rooms in a CRB design. Ten or eight replicate flowers were harvested for each variety. Postharvest experiments were two-factor designs with postharvest incubation temperatures (5, 12 and 20°C) and *A. pullulans* treatments (untreated or 10^5 c.f.u. *A. pullulans* mL^{-1}) as factors. Data were analysed as described in section 4.1.2.8. Data in text are presented as individual treatment means

5.2.2.2 *A. pullulans* preparation and application

A. pullulans (de Bary) Arnaud isolate GRAI-2 was isolated from strawberry fruit by Adikaram *et al.* (2002) and maintained on ½ strength PDA at -4°C. Flower inoculation with *A. pullulans* cells was performed according to the methods of Adikaram *et al.* (2002). Briefly, *A. pullulans* cells were obtained from 5-day old colonies. Mycelium pieces were scraped from colonies using a sterile razor blade and suspended in 5 mL portions of sterile deionized water. After briefly shaking on a rotamixer, the suspension was filtered through a double layer of sterile chessecloth and the filtrate collected. The concentration of *A. pullulans* cells in the final suspension used for glasshouse application was adjusted to 10^5 c.f.u. mL^{-1}. Preharvest spray application of *A. pullulans* was carried out until incipient run-off. Two *A. pullulans* applications, one 10 and the other 1 day before harvest, were made for each freesia variety (Lima *et al.*, 1997).

5.2.2.4 *A. pullulans* viability study after glasshouse application

The survival of the *A. pullulans* isolate on flower surfaces was assessed after application during the 2001 glasshouse trials. To assess *A. pullulans* survival, 5 treated flowers with *A. pullulans* were harvested 9 and 1 day after treatment (Schena *et al.*, 1999). Flower petals were shaken in 100 mL sterile distilled water on a rotary shaker at 150 rpm for 30 min (Schena *et al.*, 1999). The rinse water was plated on ½ PDA plates (0.1 mL $plate^{-1}$) with a pipette. The plates were incubated at 25°C and the colonies were examined after 3-4 days of incubation.

5.2.2.5 Disease assessments

Disease severity, lesion numbers, lesion diameters and senescence were recorded postharvest using the scales described in section 3.2.2.4 (Appendix 2.2, Plate A2.3).

Lesion numbers on petals were counted every day after artificial inoculation. In addition, diameters of 10 randomly selected lesions per flower were measured as described in section 3.2.2.4.

5.2.2.6 Phenylalanine ammonia lyase (PAL) analysis

PAL activity of treated var 'Dukaat' flowers was measured as a possible index of secondary metabolite synthesis after *A. pullulans* treatment. PAL activity was measured as described in section 4.1.2.7.

5.2.3 Results

5.2.3.1 Effect of preharvest *Aureobasidium pullulans* treatment

Re-isolation of *A. pullulans* applied on freesia flowers was successful 1 and 9 days after biocontrol treatment indicating that the biocontrol remained alive on flower phylloplane. *A. pullulans* was generally ineffective against *B. cinerea* independent of incubation temperature and varieties. *A. pullulans* provided slight ($P < 0.05$) protection only in the few cases (Tables 5.8, 5.9, and 5.10).

For example, on 'Cinderella' freesia flowers, *A. pullulans* significantly ($P < 0.05$) reduced disease severity when flowers were incubated at 5°C, and lesion numbers on flowers at 20°C (Tables 5.8; Appendix A5.2.1, Table A5.2.1.4). On 'Cote d'Azur' freesia flowers *A. pullulans* significantly ($P < 0.05$) reduced disease severity and lesion numbers only on flowers incubated at 5°C (Table 5.9; Appendix 5.2.1, Tables A5.2.1.12 and A5.2.1.14). Finally, in 'Dukaat' freesia flowers, *A. pullulans* significantly ($P < 0.05$) reduced lesion numbers only on flowers incubated at 12°C (Table 5.10; Appendix 5.2.1, Table A5.2.1.22).

Table 5.8: Effects of *A. pullulans* treatment on *B. cinerea* disease on freesia var. 'Cinderella' flowers. Flowers were treated with 10^5 c.f.u. mL^{-1} *A. pullulans,* inoculated with 10^4 *B. cinerea* conidia mL^{-1} and immediately incubated at 5, 12 and 20°C. Data are means of assessments on 4 successive days of incubation.

Temperature (°C)	Treatment	N [a]	Disease severity (score 0-4) [b]	Lesion number	Lesion diameter (mm)
5	Control	40	1.1 b	42 a	0.6 ab
	A. pullulans	40	0.8 a	29 a	0.6 a
12	Control	40	1.3 b	63 b	0.7 c
	A. pullulans	40	1.2 b	61 b	0.6 ab
20	Control	40	1.3 b	62 b	0.7 bc
	A. pullulans	40	1.1 b	44 a	0.7 bc

[a] Number of flower samples
[b] For each column within temperature by treatment interactions numbers followed by the same letter are not significantly different at P = 0.05.

Table 5.9: Effects of *A. pullulans* treatment on *B. cinerea* disease on freesia var. 'Cote d'Azur' flowers. Flowers were treated with 10^5 c.f.u. mL^{-1} *A. pullulans,* inoculated with 10^4 *B. cinerea* conidia mL^{-1} and immediately incubated at 5, 12 and 20°C. Data are means of assessments on 4 successive days of incubation.

Temperature (°C)	Treatment	N [a]	Disease severity (score 0-4) [b]	Lesion number	Lesion diameter (mm)
5	Control	32	1.8 b	87 b	0.9 ab
	A. pullulans	32	1.6 a	67 a	0.8 a
12	Control	32	1.9 b	122 c	1.1 b
	A. pullulans	32	1.8 b	109 c	0.9 ab
20	Control	32	1.9 b	86 b	1.0 bc
	A. pullulans	32	1.8 b	79 ab	0.9 ab

[a] Number of flower samples

[b] For each column within temperature by treatment interactions numbers followed by the same letter are not significantly different at P = 0.05.

Table 5.10: Effects of *A. pullulans* treatment on *B. cinerea* disease on freesia var. 'Dukaat' flowers. Flowers were treated with 10^5 c.f.u. mL^{-1} *A. pullulans,* inoculated with 10^4 *B. cinerea* conidia mL^{-1} and immediately incubated at 5, 12 and 20°C. Data are means of assessments on 4 successive days of incubation.

Temperature (°C)	Treatment	N [a]	Disease severity (score 0-4)[b]	Lesion number	Lesion diameter (mm)
5	Control	40	0.9 a	65 a	0.6 ab
	A. pullulans	40	0.9 a	64 a	0.6 a
12	Control	40	1.1 b	95 b	0.7 c
	A. pullulans	40	1.0 ab	73 a	0.6 ab
20	Control	40	1.0 ab	63 a	0.7 bc
	A. pullulans	40	1.1 b	62 a	0.7 bc

[a] Number of flower samples
[b] For each column within temperature by treatment interactions numbers followed by the same letter are not significantly different at P = 0.05.

A. pullulans treated flowers maintained higher PAL activities compared to unsprayed control flowers after 6h of incubation (Figure 5.3; Appendix 5.2.1, Tables A5.2.1.25–A5.2.1.28). PAL in *A. pullulans* treated flowers was slightly ($P < 0.05$) higher at 12h of incubation compared to unsprayed controls (Figure 5.3; Appendix 5.2.1, Tables A5.2.1.25–A5.2.1.28). Thereafter, PAL activities decreased for both *A. pullulans* treated and unsprayed flowers (Figure 5.3; Appendix 5.2.1, Tables A5.2.1.25–A5.2.1.28).

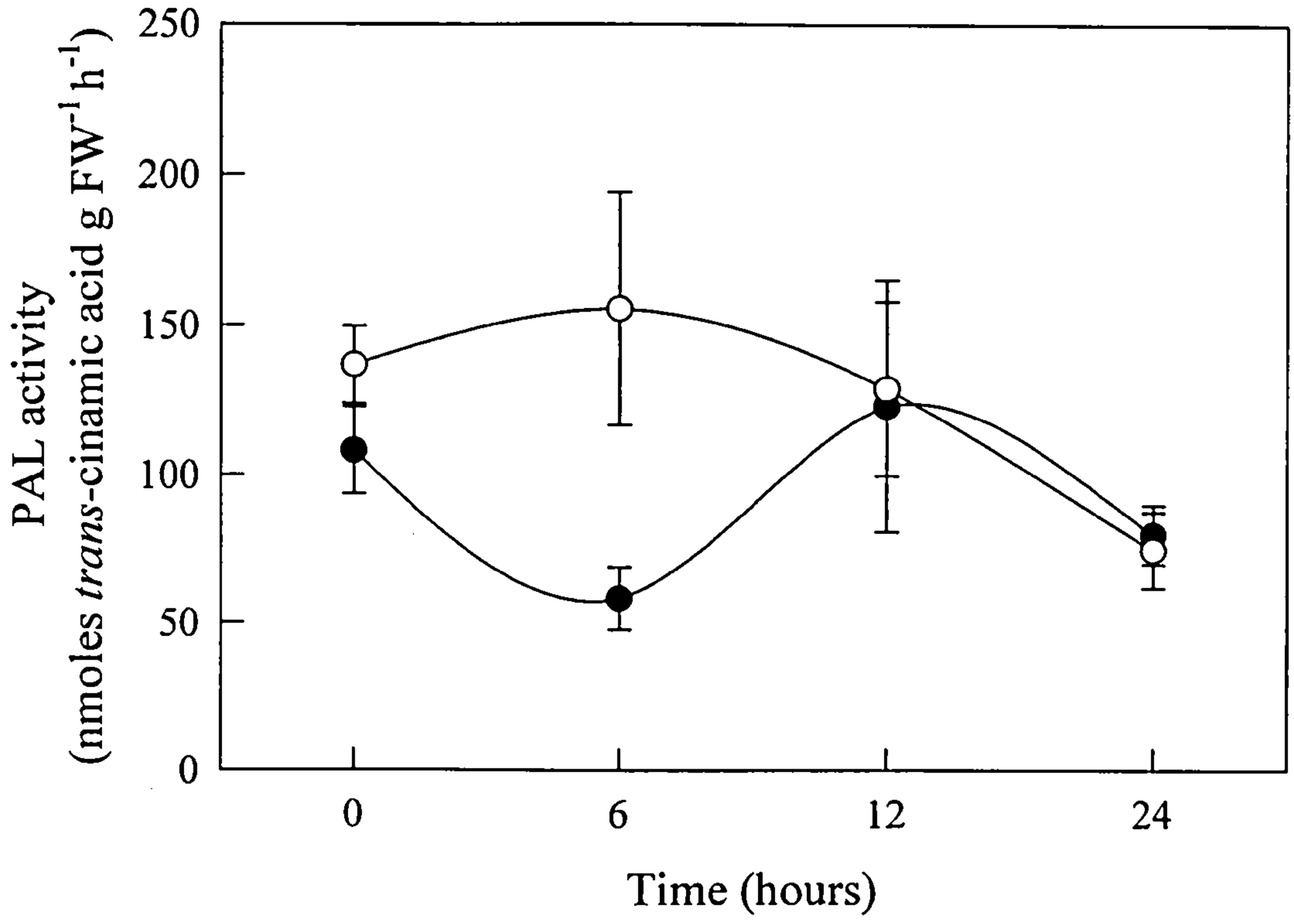

Figure 5.2: PAL activity in freesia var. 'Dukaat' flowers treated with *A. pullulans* (○), or left untreated (●). Data are means of three replicates each consisted of 3 three.flowers. Bars indicate the SE of each treatment (n = 3).

5.2.4 Discussion

A. pullulans had a very limited effect on suppressing *B. cinerea* on cut freesia flowers. *B. cinerea* disease reduction upon *A. pullulans* treatment ranged between 11 on disease severity of 'Dukaat' flowers incubated at 5°C and 29%, on lesion number on 'Cinderella' flowers incubated at 20°C. In contrast, *A. pullulans* was antagonistic towards *B. cinerea* on strawberry fruits (Lima *et al.*, 1997), table grapes and apple fruits (Castoria *et al.*, 2001) and on cherry tomatoes (Schena *et al.*, 1999). However, on these studies *A. pullulans* was applied in very high concentrations of 1 x 10^8 c.f.u. L^{-1}. According to Droby *et al.* (1989) and Ippolito and Nigro (2000b), improved antagonistic

activity is biocontrol concentration-dependent and can be reserved by the addition of exogenous nutrients.

PAL activity in *A. pullulans* treated freesia flowers was slightly ($P < 0.05$) higher than the unsprayed control after 6h of incubation. Thus, there is an indication that *A. pullulans* slightly induced defence mechanisms. Suppression of postharvest *B. cinerea* infection by *A. pullulans* in association with induction of defence responses is a prospect (Ippolito *et al.*, 2000a; Castoria *et al.* 2001; Adikaram *et al.*, 2002). Green strawberry fruit treated with *A. pullulans* had greater antifungal activity than untreated controls (Adikaram *et al.*, 2002). Extracellular exo-chitinase and β-1,3-glucanase activities were detected in apple wounds treated with 6×10^7 *A. pullulans* (Ippolito *et al.*, 2000a) and both *in-vitro* and in apple wounds treated with 30 mL (1×10^8 cells mL^{-1}) *A. pullulans* (Castoria *et al.*, 2001).

According to the results of the present study, *A. pullulans* alone could not give the level of control associated with synthetic fungicides (section 5.1). However, future research should continue to investigate whether *A. pullulans* can play a role in IPM systems. For instance, combination of *A. pullulans* treatments with fungicides or plant activators might offer synergistic benefits for postharvest *B. cinerea* disease control (Wilson and El Ghaouth, 1993).

5.3 METHYL JASMONATE PREHARVEST TREATMENTS

5.3.1 Introduction

JA and its methyl ester are chemical activators that can act separately to the SA-dependant pathway (section 2.5.7.1). These compounds have been used in a variety of crops (section 2.6.3.4). JA and its methyl ester (MeJA) applied as foliar sprays to potato (cv. Bintje or Alpha) and tomato (cv. Baby) plants protected them against infection with *P. infestans* (Cohen *et al.*, 1993). Treatment of *Arabidopsis* plants with MeJA reduced *A. brassicicola, B. cinerea* and *Plectosphaerella cucumerina* disease (Thomma *et al.*, 2000). MeJA and acibenzolar were tested against bacterial and insect attacks on tomato field

grown plants (Thaller *et al.*, 1999). Two signaling pathways, one involving SA-dependant and another JA-dependant, were proposed to provide resistance against a bacterial speck disease caused by *Pseudomonas syringae* pv. *tomato* and against the larvae of the beet armyworm *Spodoptera exigua*, respectively.

The present study investigates the efficacy of MeJA applied as a preharvest foliar spray against postharvest infection by *B. cinerea* of cut freesia flowers.

5.3.2 Materials and methods

5.3.2.1 Plant material, glasshouse and postharvest designs and statistical analysis

Freesia vars 'Cote d'Azur' and 'Dukaat' were used in the MeJA glasshouse trial in 2002. Steam sterilised soil was transported by truck from Zwetsloots & Sons Ltd. to Cranfield University at Silsoe (Plate 5.2).

Freesia corms were arranged in a CRB design with chemical treatments and freesia variety as factors. A single 16 m long x 1.10 m wide bed was divided into plots of two varieties each treated with four MeJA concentrations. All treatments, totally eight, were replicated twice. Thus, the bed was divided in 16 equal-sized plots. Ten replicate flowers per treatment were harvested on the same day (morning) at the commercial harvest stage with all buds still closed (Appendix 2.2, Plate A2.2) and used for postharvest disease assessments. After harvest, flowers were arranged inside incubation rooms in CRB design with factors of temperatures (5, 12 and 20°C) and MeJA treatment (0, 200, 400 and 600 μM). Data were analysed as described in section 4.2.1.8. Data are presented as main factor means in tables and the corresponding treatment means and interactions in figures.

Plate 5.2: Freesia vars. 'Cote d'Azur' and 'Dukaat' plants arranged in a CRB design on a flower-bed inside a glasshouse at Cranfield University at Silsoe.

5.3.2.2 Methyl jasmonate preharvest chemical treatment

MeJA application started 28 days before harvest (Thaller *et al.*, 1996). Thereafter, MeJA was applied every 7 days until harvest giving four sprays in total for each freesia variety. Each variety was treated with MeJA at 0 (control), 200, 400 and 600 μM (Meir *et al.*, 1998) prepared as described in section 4.2.2.4.

5.3.2.3 Postharvest inoculation and incubation

After harvest, flowers were taken in the laboratory and inoculated with a *B. cinerea* suspension of 10^4 conidia mL^{-1} as described in section 3.2.2.2. After inoculation, flowers were covered with plastic transparent bags to maintain *ca.* 100% RH (section .4.1.2.3). They were then placed at 5, 12 and 20°C for incubation. Disease assessments commenced 24h after inoculation and carried out for 4 days.

5.3.2.4 Disease assessments

Disease severity score, lesion numbers, lesion diameters and senescence were recorded on the harvested flowers using the scales described in section 3.2.2.4 (Appendix 2.2, Plate A2.3). Lesion numbers on petals were counted every day after artificial inoculation. Diameters of 10 randomly selected lesions per flower were measured every day as described in section 3.2.2.4.

5.3.3 Results

5.3.3.1 Effect of preharvest methyl jasmonate treatment

B. cinerea disease severity, lesion numbers and lesion diameters were variety, temperature and MeJA treatment dependent. In detail, *B. cinerea* disease severity, lesion numbers and lesion diameters on var. 'Cote d'Azur' freesia flowers increased with increasing incubation temperature (Table 5.11). Between MeJA concentration only slight ($P > 0.05$) differences were recorded. MeJA treatment at 400 μM significantly ($P < 0.05$) reduced disease severity, lesion numbers and lesion diameters compared to untreated controls (Table 5.11). In general, freesia var. 'Cote d'Azur' flowers were more susceptible to *B. cinerea* infection compared to 'Dukaat' flowers (Table 5.11 and 5.12). Main factor means of disease severity and lesion numbers on freesia var. 'Dukaat' flowers were significantly ($P < 0.05$) higher at 12°C (Table 5.12). All MeJA treatments moderately ($P < 0.05$) reduced disease severity, lesion numbers and lesion diameters on 'Dukaat' main factor means compared to untreated controls (Table 5.12).

Individual MeJA treatments were more effective independently of the variety tested for flowers incubated at 20°C compared to untreated controls (Figures 5.3 and 5.4). Lesion numbers on 'Dukaat' freesia flowers incubated at 20°C were reduced by up to 55 % after MeJA treatment (Figure 5.4). However, for freesia var. 'Cote d'Azur' and 'Dukaat' flowers incubated at 12°C no significant ($P > 0.05$) reduction in disease severity lesion number and lesion diameter was observed except in the few individual cases. In detail, freesia var. 'Cote d'Azur' flowers treated with MeJA at 200 and 400 μM significantly ($P < 0.05$) reduced disease severity lesion number and lesion diameter at 20°C compared to untreated controls (Figure 5.3, Appendix 5.3.1, Tables A5.3.1.4, A5.3.1.6 and A5.3.1.8). Freesia var. 'Dukaat' flowers treated with 400 and 600 μM MeJA markedly reduced disease severity and lesion numbers at 20°C compared to untreated controls. Also, freesia var. 'Dukaat' flowers treated with MeJA at 200 and 400 μM significantly ($P < 0.05$) reduced lesion diameters compared to untreated controls (Figure 5.3, Appendix 5.3.1, Tables A5.3.1.12, A5.3.1.14 and A5.3.1.16). MeJA was

ineffective in reducing *B. cinerea* disease severity, lesion number and lesion diameter in 'Cote d'Azur' and 'Dukaat' flowers incubated at 5°C.

Table 5.11: Effects of preharvest treatments with MeJA on *B. cinerea* disease on freesia var. 'Cote d'Azur' flowers. Flowers were sprayed with 0 (control), 200, 400 and 600 μM MeJA, starting 28 days before harvest. Then, flowers were artificially inoculated postharvest with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 4 days at 5, 12 and 20°C. Disease assessments were carried out daily over 4 days of incubation. Corresponding individual treatment means are presented in Figure 5.3.

Factors	**Disease variables**		
	Disease severity (score 0-4) [a]	**Lesion number**	**Lesion diameter (mm)**
1) Temperature (°C) [b]			
5	0.5 a	16 a	0.5 a
12	2.5 b	98 b	0.9 b
20	2.5 b	105 b	0.9 b
2) MeJA concentration (μM)			
0	2.1 b	79 b	1.0 c
200	1.9 ab	79 b	0.7 ab
400	1.6 a	63 a	0.7 b
600	1.7 a	69 a	0.6 a

[a] Data are main factor means of disease severity, lesion number and lesion diameter.
[b] Within main factor means, numbers followed by the same letter are not significantly different at P = 0.05.

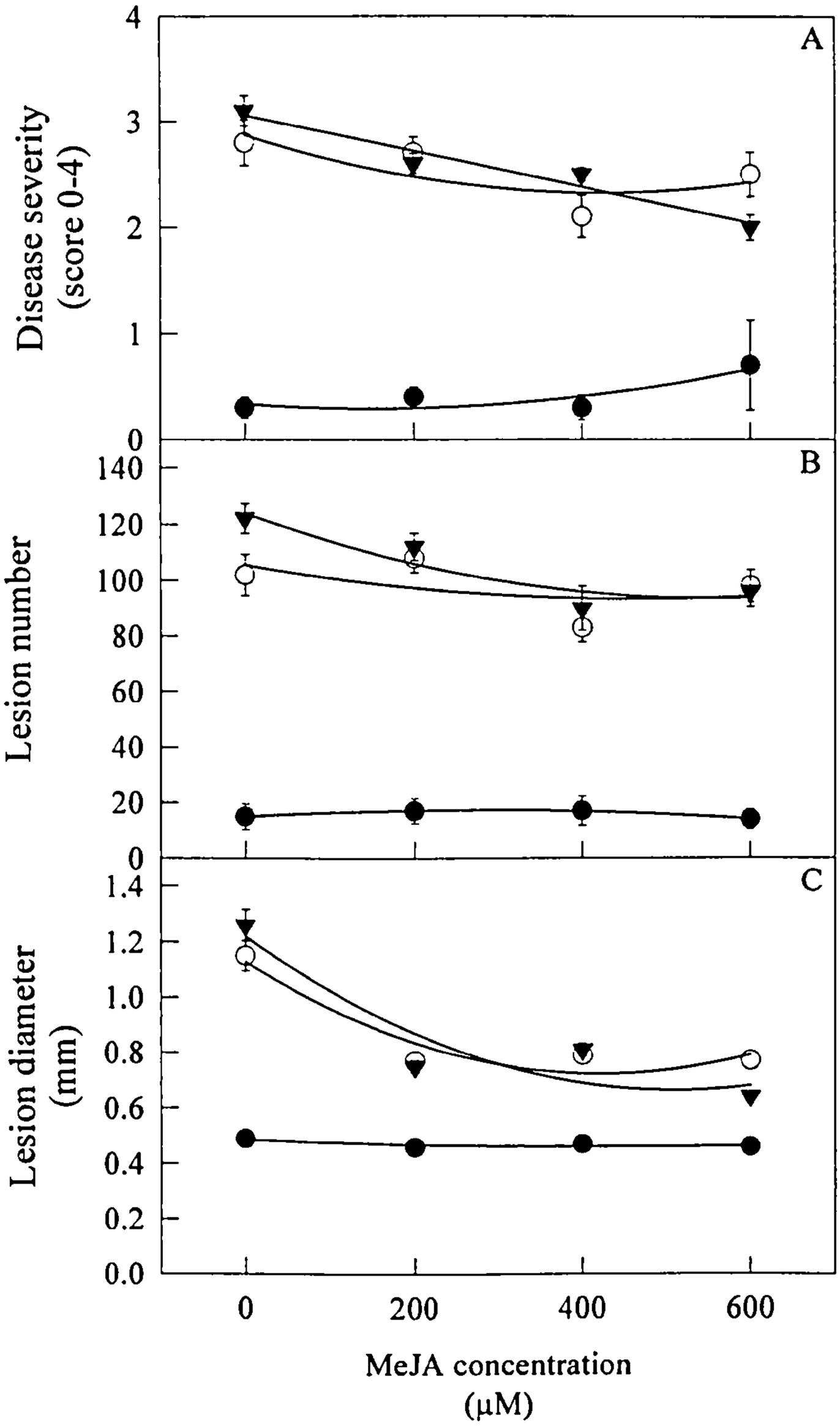

Figure 5.3: Quadratic regressions of individual treatment means for disease severity (A), lesion number (B) and lesion diameter (C) for freesia var. 'Cote d'Azur' flowers sprayed with 0 (control), 200, 400 and 600 μM MeJA starting 28 days before harvest. Then flowers were inoculated postharvest with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5 (●), 12 (○) and 20°C (▼). Each data point represents the mean of assessments on each of 4 sequential days of incubation. Bars indicate the SE for each treatment (n = 40). Main factor means are presented in Table 5.11 and regression parameters in Appendix 5.3.1, Table A5.3.1.9.

Table 5.12: Effects of preharvest treatments with MeJA on *B. cinerea* disease on freesia var. 'Dukaat' flowers. Flowers were sprayed with 0 (control), 200, 400 and 600 μM MeJA, starting 28 days before harvest. Then, flowers were artificially inoculated postharvest with 10^4 *B. cinerea* conidia mL^{-1} and incubated for 4 days at 5, 12 and 20°C. Disease assessments were carried out daily over 4 days of incubation. Corresponding individual treatment means are presented in Figure 5.4.

Factors	**Disease variables**		
	Disease severity (score 0-4) [a]	**Lesion number**	**Lesion diameter (mm)**
1) Temperature (°C) [b]			
5	0.3 a	16 a	0.5 a
12	1.1 c	38 c	0.7 b
20	0.7 b	27 b	0.7 b
2) MeJA concentration (μM)			
0	0.9 b	34 b	0.8 c
200	0.6 a	23 a	0.6 a
400	0.6 a	23 a	0.6 a
600	0.7 a	28 a	0.6 b

[a] Data are main factor means of disease severity, lesion number and lesion diameter.
[b] Within main factor means, numbers followed by the same letter are not significantly different at P = 0.05.

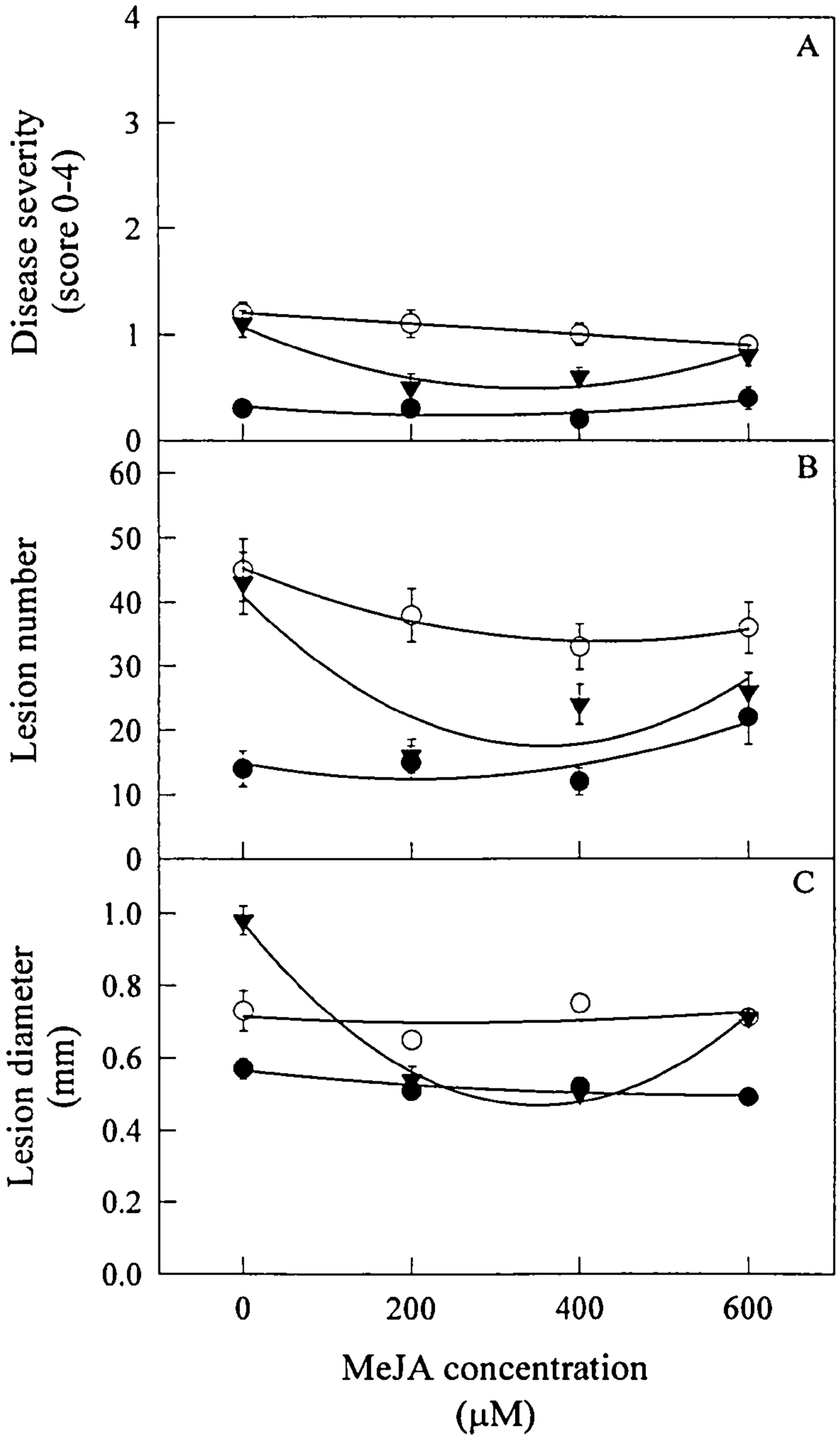

Figure 5.4: Quadratic regressions of individual treatment means for disease severity (A), lesion number (B) and lesion diameter (C) for freesia var. 'Dukaat' flowers sprayed with 0 (control), 200, 400 and 600 μM MeJA starting 28 days before harvest. Then flowers were inoculated postharvest with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5 (●), 12 (○) and 20°C (▼). Each data point represents the mean of assessments on each of 4 sequential days of incubation. Bars indicate the SE for each treatment (n = 40). Main factor means are presented in Table 5.12 and regression parameters in Appendix 5.3.1, Table A5.3.1.17.

5.3.4 Discussion

In the present MeJA glasshouse trial, the efficacy of MeJA was temperature and variety dependent. MeJA treatment was highly effective in suppressing *B. cinerea* disease when flowers were incubated at 20°C. In addition, MeJA was more effective for 'Dukaat' compared to 'Cote d'Azur' flowers. MeJA treated freesia var. 'Dukaat' flowers at 200μM incubated at 20°C showed reductions up to 56, 61, and 49% in disease severity, lesion number and lesion diameter compared to controls, respectively. In contrast, MeJA treated 'Cote d'Azur' flowers at 600μM incubated at 20°C showed a reduction up to 36, 26, and 49% in disease severity, lesion number and lesion diameter compared to controls, respectively. Different protection levels after MeJA treatment in different varieties have been reported by Cohen *et al.* (1993) for potatoes and by Meir *et al.* (1998) for cut roses. In 'Bintje' and 'Alpha' potato cultivars, MeJA provided different protection levels after challenge with *Phytophthora infestans.* Treatment with MeJA at 125 μg mL^{-1}, provided approximately 50% disease reduction on cv. Bintje but only 20% on cv. Alpha potato plants compared to untreated controls (Cohen *et al.,* 1993).

Overall, MeJA treatment of freesias could provide a higher level of protection against *B. cinerea* when applied preharvest compared to acibenzolar (section 5.1). The JA-dependant pathway is probably the key pathway regulated upon *B. cinerea* infection (Thomma *et al.,* 1998; Govrin and Levine, 2002; section 2.5.7.2). MeJA was able to provide significant protection against *B. cinerea* infection of cut freesia flowers. Therefore it could be considered as a potential tool for IPM systems. However, two factors limiting MeJA efficacy should be addressed before commercial application. Firstly, as shown in all MeJA postharvest experiments of this thesis, the efficacy of MeJA is strongly dependant upon incubation temperature (sections 4.2.3.1, 4.2.3.4, 4.2.3.7, and 4.4.3). MeJA losses its ability to induce the JA-dependant defence responses under low storage temperature. Secondly, the efficacy of MeJA differs between freesia variety as presented in section 5.3.3.

5.4 GENERAL CONCLUSIONS

Elicitor treatments of freesia plants before harvest could potentially enhance defence mechanisms and result in more disease resistant freesia flowers after harvest. However, acibenzolar-S-methyl was only effective in selected treatments and conditions. There was a difference in results between the years 2001 and 2002. Firstly, disease levels in 2002 were much lower under all three temperature conditions compared with those in 2001. This difference was also independent of variety tested. In the acibenzolar glasshouse trial in 2002, flowers were generally more resistant to postharvest infection by *B. cinerea.* Disease was significantly reduced in both 'Cote d'Azur' and 'Dukaat' freesia flowers especially when incubated at 20°C. However, it was unclear whether acibenzolar induced systemic and/or local defence responses. Biochemical investigations in 2001 suggested that acibenzolar did not induce PAL activity. Thus, it was unclear whether SAR induction contributed at all to *B. cinerea* disease reduction on freesia flowers. Thomma *et al.* (1998) and Govrin and Levine (2002) have shown that plants deficient in the SA-dependent defence response (e.g. *npr1* and *NahG*) do not show enhanced susceptibility towards *B. cinerea.* Accordingly, SA might not be essential for inhibiting *B. cinerea* on fressia flowers. Similar observations were reported by Friedrich *et al.* (1996). According to Friedrich *et al.* (1996), acibenzolar did not suppress *B. cinerea* on tobacco plants.

MeJA conferred markedly systemic protection compared to other elicitors tested. However, MeJA effectiveness was both variety and temperature dependant. MeJA provided protection on freesia flowers by reducing disease severity, lesion numbers, and lesion diameters. The difference in protection levels conferred by acibenzolar versus MeJA treatment of freesia flowers might be attributed to the different SA- and JA-dependant pathways regulated upon infections by different pathogens (Pieterse and van Loon, 1999; Thomma *et al.,* 2001). For example, in *Arabidopsis*, SA- and JA-dependent pathways were up-regulated upon infection to different types of fungal pathogens (section 2.3.2.7). *B. cinerea* is probably only suppressed after JA-dependant pathway activation (Pieterse and van Loon, 1999; Thomma *et al.,* 2001; Govrin and Levine, 2002).

CHAPTER 6

OVERALL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Postharvest infection of freesia flowers by *B. cinerea* has concerned growers and sellers over the years (D. Zwetsloot pers. comm., 2000). This is one of the first studies on the problem. The work presented in this thesis has demonstrated potential to improve control of *B. cinerea* specking on cut freesia flowers via implementation of integrated disease management strategies based on induction of natural resistance mechanisms. This prospect has been elaborated in a diagramatic model by Joyce and Johnson (1999). Enhancement of freesia flowers resistance was achieved to varying degrees by both pre- and postharvest treatments with acibenzolar-S-methyl, methyl jasmonate and UV-C irradiation. However, considerable research is required for maximum manipulation of natural disease resistance.

6.1 POSTHARVEST INFECTION OF CUT FREESIA FLOWERS BY *B. CINEREA*

The epidemiology of *B. cinerea* infection was investigated with a view to discerning a relationship between preharvest environmental conditions and postharvest freesia rejections. *B. cinerea* incidence seemingly followed a seasonal pattern in 2000, similar to that found on gerbera and rose flowers by Kerssies (1993) and Kerssies *et al.* (1995), respectively. Higher proportions of freesia flowers were rejected due to *B. cinerea* infection during spring (April-May), early summer (June) and autumn (October) than over the rest of the year (section 3.1). Environmental conditions monitored inside and outside a freesia glasshouse in The Netherlands, showed no obvious correlations that explained the seasonal effects between the glasshouse environmental conditions and *B. cinerea* disease incidence as recorded by the rejections in the UK in 2000.

Histological studies showed that the necrotic lesions produced at 20°C by *B. cinerea* on freesia petals, had a volcano-like shape and were similar to those reported on rose by Pie and De Leeuw (1991) (section 3.2). Infection of freesia flowers by *B.*

cinerea, was also similar to that reported by Salinas and Verhoeff (1995) on gerbera petals. The *B. cinerea* infection process on freesia petals was monitored in conjuction with factors (viz. temperature, RH and inoculum level) thought likely to affect *B. cinerea* disease incidence. Temperature was not a limiting factor for freesia specking by *B. cinerea.* Postharvest incubation temperatures of both 5 and 20°C resulted in disease. Similar results were reported over a wide temperatures range of 4-25°C by Salinas *et al.* (1989) for *B. cinerea* lesion formation on gerbera flowers. In contrast, RH was an important factor in infection of freesia flowers by *B. cinerea.* The results of the present study also showed that the time needed for freesia petals to become infected by *B. cinerea* was less than 24h at 12°C and 80-90% RH. Such conditions commonly occurred during freesia transportation (section 3.1.3). The time taken for freesia flowers to be transported from The Netherlands to the UK is typically sufficient for infection of both gerbera (Salinas *et al.* 1989) and freesia flowers. Therefore, the development of *B. cinerea* lesions on freesia flower petals could conceivably occur during the first 24 to 65h of postharvest handling in transit from flower auction in The Netherlands to the wholesaler in the UK. This scenario is especially likely under RH levels ranging between 80-100% or in the presence of a water film on petal surfaces and at temperatures ranging from 4-25°C. At saturated RH (100%) disease symptoms can be visible within only 24h of constant temperature of 5°C. More research is required on handling chain conditions from The Netherlands to the UK. A model that combines the effects of temperature and RH over time may be created in view to further the understanding of the timing and function of *B. cinerea* infection. Moreover, additional scanning electron microscopy and immunohistological studies could provide more detailed information on the freesia-*B. cinerea* infection process.

6.2 EFFECT OF PRE- AND POSTHARVEST APPLICATION OF ACIBENZOLAR-S-METHYL AND METHYL JASMONATE IN SUPPRESSING *B. CINEREA* ON CUT FREESIA FLOWERS

For the first time, the plant activators acibenzolar-S-methyl and MeJA were tested as potential postharvest elicitor treatments for freesia flowers. Management of postharvest infection by *B. cinerea* is difficult due to pathogen's ability to grow under low temperature and high relative humidity conditions (Jarvis, 1977). Interest in plant activators has emerged in line with growing public demand for socio-environmentally sound methods for disease management (Jacobsen and Backman, 1993). Acibenzolar was somewhat effective compared to untreated controls mostly when applied at 0.15 g AI L^{-1}. Howver, acibenzolar applied as a postharvest treatment only provided limited protection against *B. cinerea* on freesia flowers (section 4.1). PAL assays of freesia flowers did not indicate that acibenzolar induced postharvest SAR or other PAL-related responses. Postharvest acibenzolar application might not be as effective in detached organs due to changes in their physiology and/or may be too late in plant organ development to induce systemic responses that could potentially lead to *B. cinerea* disease suppression.

Acibenzolar applied preharvest was more effective than postharvest application in reducing *B. cinerea* disease severity on 'Cinderella', 'Cote d'Azur' and 'Dukaat' freesia flowers (section 5.1). *In-vitro* studies showed direct antifungal activity of acibenzolar against *B. cinerea* colony growth and conidial germination (section 4.2.3.10). This observation agrees with that of Terry and Joyce (2000), but contradicts previous findings by Friedrich *et al.* (1996) and Kessmann *et al.* (1996). Lesion numbers and size were generally reduced at all incubation temperatures, but not consistently over concentration, incubation temperature and variety. Inconsistency of acibenzolar effects may be explained by: 1) variability of environmental conditions throughout the 3-year glasshouse trials period which may have affected defence enhancement (Herms and Mattson, 1992; Terry, 2002); and, 2) infection by *B. cinerea* is not necessarily sensitive to induced SAR responses, and thus acibenzolar treatments may not suppress *B. cinerea* (Thomma *et al.*, 1998; Govrin and Levine, 2002). Friedrich *et al.* (1996) reported that acibenzolar failed

to induce systemic protection against *B. cinerea* in tobacco, but worked against other pathogens. The apparent inability of acibenzolar to induce SAR against *B. cinerea* is seemingly supported by the observation that PAL activity in acibenzolar treated flowers was not higher compared to untreated controls. Therefore, acibenzolar treatment apparently did not induce biochemical defence processes, such as the production of antifungal secondary metabolites like phytoalexins through the phenylpropanoid pathway (Kombrink and Somssich, 1995; Kuć, 1995).

In contrast to inconsistent effects of acibenzolar applied postharvest, MeJA was markedly effective in suppressing *B. cinerea* specking on cut freesia flowers when applied either pre- or postharvest (sections 4.2, 4.3, 4.4 and 5.3). MeJA effectiveness was application method and concentration dependent. MeJA applied as gas was more effective compared to pulsing or spraying. It is possible that methyl jasmonate may function as an airborne signal which activates disease resistance and the expression of defence related genes in plant tissue (Shulaev *et al.*, 1997). This finding agrees with earlier findings in *Arabidopsis* by Thomma *et al.* (2000). Gaseous MeJA treatments markedly reduced lesion diameters caused by *B. cinerea*. In *Arabidopsis*, this effect was mediated via the JA-dependent defence responses (Thomma *et al.*, 2000). MeJA did not exert any direct antifungal activity *in-vitro* except at 600 μM (section 4.2.3.10). It is likely that MeJA treatment also on freesia flowers reduced *B. cinerea* disease by inducing defence responses probably correlated with JA-dapendent pathway. PPO levels in freesia flowers increased after gaseous MeJA treatment by 47 and 57% as compared to untreated controls, with or without *B. cinerea* (section 4.3). However, PAL activity in MeJA treated freesia flowers decreased markedly compared to controls after 12h and was maintained at this level. The abrupt decrease in PAL activity was greater when MeJA treated flowers were artificially inoculated with *B. cinerea*. Overall, these findings suggest that MeJA treatment might suppress the action of PAL in the phenylpropanoid pathway and consequently reduce or block SA production. Antagonistic regulation of JA- and SA- dependent pathways has been documented in the past by Pena-Cortes *et al.* (1993), Conconi *et al.* (1996), Niki *et al.* (1998), Gupta *et al.* (2000), and Rao *et al.* (2000). The apparent suppression of PAL in freesia flowers by MeJA might constitute additional evidence of a JA- and SA- antagonistic effect.

MeJA applied to freesia plants 28 days before harvest (section 5.3) suppressed postharvest flower specking caused by *B. cinerea* in both a temperature and variety dependent fashion. MeJA treatment was highly effective when flowers were incubated at 20°C compared to incubation at 5 or 12°C. It is likely that low incubation temperatures slow down the plant's metabolism and also the production of defence related compounds (Jarvis, 1980b). MeJA was more effective for 'Dukaat' compared to 'Cote d'Azur' flowers. According to Cohen *et al.* (1993) different levels of protection were conferred on 'Bintje' versus 'Alpha' potato cultivars treated with MeJA and then challenged with *Phytophthora infestans.* Overall, however, MeJA treatment provided a considerable level of protection against *B. cinerea* when applied preharvest and, thus, could be considered a promising tool in an integrated disease management context. Further study at the molecular level is warranted to help interpret the MeJA mode of action in freesia flowers. Also, additional *in-planta* trials on additional freesia varieties and a wider range of MeJA concentrations may help in better understanding MeJA efficacy.

In view of the promising results using MeJA, it is likely that elicitor based strategies within integrated disease management (IPM) could be used for *Botrytis* on freesias. In turn, IPM might minimise the risk of *B. cinerea* developing resistance to fungicides and also reduce public concerns over extensive fungicide use (Jacobsen and Backman, 1993). The present work has demonstrated the potential usefulness of elicitors. More research is now required on the ability of MeJA to induce systemic responses (e.g. phytoalexins, PR-proteins, other defence related compounds). However, as gaseous MeJA gave the best postharvest disease suppression at 20°C, more research should be undertaken on the integration of gaseous MeJA treatments in the freesia handling chain. Subsequently, gaseous MeJA could be included in commercial postharvest disease management practices for freesia flowers.

More research should now be undertaken into potential synergistic effects of combined pre- and postharvest treatments with plant activators and/or abiotic biological agents (i.e. UV-C irradiation). In due course, pre and/or postharvest use of plant activators could have commercial potential for postharvest disease suppression (Kessmann *et al.*, 1994; Kessmann *et al.*, 1996; Thaller *et al.*, 1996; Meir *et al.*, 1998; Huang *et al.*, 2000).

6.3 UV-C IRRADIATION OF CUT FREESIA FLOWERS AGAINST POSTHARVEST INFECTION BY *B. CINEREA*

UV-C irradiation was effective in suppressing flower specking caused by *B. cinerea* when applied after artificial inoculation (section 4.5). UV-C irradiation on pre-inoculated freesia petals apparently directly affected *B. cinerea*. UV-C irradiation is considered a potent tool for *B. cinerea* conidia inactivation. The direct germicidal UV-C effect has been reported earlier by others including Islam *et al.* (1998) and Marquenie *et al.* (2002) *in-vitro* and Stevens *et al.* (1998) on peaches. Attempts to induce disease resistance in cut freesia flowers with UV-C irradiation were possibly successful but only in few cases. For example, UV-C irradiation before artificial inoculation at 0.5 and 1 kJ m^{-2} significantly ($P < 0.05$) reduced *B. cinerea* disease severity and lesion numbers compared to non-irradiated controls. However, published works have shown that low doses of UV-C irradiation can induce defence responses in harvested fruits and vegetables (Liu *et al.*, 1993; Stevens *et al.*, 1996; Nigro *et al.*, 1998; Gonzalez-Aguilar *et al.*, 2001). Therefore, it is essential to continue investigations on most effective irradiation doses, which will provide disease suppression without affecting flower quality. Also, more research is required to integrate UV-C irradiation into the commercial postharvest disease management of freesia flowers. UV-C irradiation might be applied to different places throughout the freesia postharvest handling chain offering a significant level of protection against *B. cinerea* specking.

6.4 PREHARVEST *A. PULLULANS* APPLICATION ONTO FREESIA CROPS TO SUPPRESS POSTHARVEST INFECTION BY *B. CINEREA*

Treatment with *Aureobasidium pullulans* had little effect in suppressing *B. cinerea* on cut freesia flowers (section 5.3). Nonetheless, PAL activity in *A. pullulans* treated freesia flowers was seemingly maintained higher than in untreated controls over the first 6h of incubation. This difference in PAL activity may suggest possible induction of defence mechanisms which, however, were not sufficient to suppress *B. cinerea* infection. Induction of defence responses in freesia flowers by *A. pullulans* treatment

agrees with earlier findings of apple fruit or *in-vitro* (agar plates) where raised chitinase and β-1,3-glucanase activity was reported (Ippolito *et al.*, 2000a; Castoria *et al.*, 2001). Induction of phytoalexins and boosting of preformed antifungal compounds was also found after *A. pullulans* treatment of green strawberry fruits (Adikaram *et al.*, 2002). In view of promising results in these other systems, further investigations are merited for freesia. If promise is shown in due course, then biocontrol treatments need to be optimised. Nevertheless, in order to optimise *A. pullulans* efficacy in the field more research is required on the adaptability of biocontrol agents under various environmental conditions (i.e. low-nutrient availability on the phylloplane, UV irradiation and temperature extremes) (Dik and Elad, 1999; Ippolito and Nigro, 2000).

Cut freesias are economically important flowers grown systematically in many parts of Europe including The Netherlands and the UK. This study was the first mention of cut freesia flowers infection by *B. cinerea.* This study has provided valuable insights into the factors affecting postharvest infection of freesia flowers by *B. cinerea* and into alternative methods for *B. cinerea* disease management. However, more research is required to improve understanding of the freesia-*B. cinerea* interactions. Also, more research is needed on the integration of the potential plant activators used in the present studies into an IPM system.

REFERENCES

Acock, B., and Nichols, R. 1979. Effects of sucrose on water relations of cut, senescing, carnations-flowers. *Annals of Botany* **44**: 221-230

Adair, C.N. 1971. Influence of controlled-atmosphere storage conditions of cabbage postharvest decay. *Plant Disease Reporter* **55**: 864-868

Adikaram, N.K.B., Joyce, D.C., and Terry, L.A. 2002. Biocontrol activity and induced resistance as a possible mode of action for *Aureobasidium pullulans* against grey mould of strawberry fruit. *Austalasian Plant Pathology* **31**: 223-229

Agrios, G. N. 1997. *Plant Pathology* (4th edition). London UK. Academic Press

Alam, S., Joyce, D., and Wearing, A. 1996. Effects of equilibrium humidity on in vitro growth of *Botrytis cinerea* and *Alternaria alternata*. *Australian Journal of Experimental Agriculture* **36**: 383-388

Amariutei, A., Burxo, I., and Cracium, C. 1995. Some physiological and ultrastructural changes of freesia cut flowers treated with silverthiosulphate. *Acta Horticulturae* **368**, *International Symposium on Postharvest Treatments of Horticultural Crops* (abstract)

Anfoka, G.H., 2000. Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum*. Mill cv. Vollendung) to Cucumber mosaic virus. *Crop Protection* **19**: 401-405

Anonymous, 1960. Index of plant disease in the United States. Crop Research Division Agricultural Research Service United States Department of Agriculture, Agriculture Handbook No. 165 pp. 226-227

Anonymous, 2001. Cut flowers and foliage (EU market survey, 2001). Compiled by CBI and J. Lamming. www.cbi.nl/downloads/cut_flowers/survey_cut_flowers_2001.pdf. 9/2/03

Atkinson, M.M. 1993. Molecular mechanisms of pathogen recognition by plants. *Advances in Plant Pathology* **10**: 35-64

Barden, L.E., and Hanan, J.J. 1972. Effect of ethylene on carnation keeping life. *Journal of the American Society for Horticultural Science* **97**: 785-788

Bary, A., de. 1884. Vergleichende morphologie und biology der pilze. Engelmann, Leipsig. In "*Botryotinia* and *Botrytis* species: taxonomy, physiology, and pathogenicity. A guide to the literature". (Ed. Jarvis, W.R.) 1977 Research Branch Canada Department of Agriculture, Monograph No 15

Beaumont, A., Dillon Weston, W.A.R, and Wallace, E.R. 1936. Tulip fire. *Annals of Applied Biology* **23**: 57-88

Beier, R.C., and Oertli, E.H. 1983. Psoralen and other linear furocoumarins as phytoalexins in celery. *Phytochemistry* **22**: 2595-2597

Benhamou, N., and Bélanger, R.R. 1998. Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum* f.sp. *radicis-lycopersici* in tomato. *Plant Physiology* **118**: 1203-1212

Benito, E.P., ten Have, A., van 't Klooster, J.W., and van Kan J.A.L. 1998. Fungal and plant gene expression during synchronized infection of tomato leaves by *Botrytis cinerea*. *European Journal of Plant Pathology* **104**: 207-220

Ben-Yehoshua, S.B., Rodov, V., Kim, J.J., Carmeli, S. 1992. Preformed and induced antifungal materials of citrus fruits in relation to the enhancement of decay resistance by heat and ultraviolet treatments. *Journal of Agricultural Food Chemistry* **40**: 1217-1221

Beyer, E.M. 1976. Silver ion: a potent antiethylene agent in cucumber and tomato. *HortScience* **11**: 195-196

Blakeman, J.P. 1980. Behaviour of conidia on aerial plant surfaces. In 'The Biology of *Botrytis*' Eds. Coley-Smith, J.R., Verhoeff, K., and Jarvis, W.R. Academic Press, York, London pp. 115-148.

Blankenship, S.M., and Dole, J.M. 2003. 1-methylcyclopropene: a review. *Postharvest Biology and Technology* **28**: 1-25

Boesewinkel, H.J. 1976. Storage of fungal cultures in water. *Transactions of British Mycological Society* **66**: 183-185

Boff, P., Kohl, J., Gerlagh, M., and De Kraker, J. 2001. Biocontol of grey mould by *Ulocladium atrum* applied at different flower and fruit stages of strawbery. *BioControl* **47**: 193-206

Bokshii, A., Morris, S., Deverall, B., and Stephens, B. 2000. Indction of systemic acquired resistance in potato. *Australian Potato Research, Development and Technology Transfer Conference, Adelaide, Australia, 31 July – 2 August*

Borochov, A., Halevy, A.H., Borochov, H., and Shinitzky, M. 1978. Microviscosity of rose petal's plasmalemma as affected by age and environmental factors. *Plant Physiology* **61**: 812-815

Bostock, R., Kuć, J., and Laine, R. 1981. Eicosapentaenoic and arachidonic acids from *Phytophthora infestans* elicit fungitoxic sesquiterpenes in potato. *Science* **212**: 67-69

Bowell, G.P. 1999. Role of active oxygen species and NO in plant defence responses. *Current Opinion in Plant Biology* **2**: 287-294

Brisset, M-N., Cesbron, S., Thomson S.V., and Paulin, J-P. 2000. Acibenzolar-S-methyl induces the accumulation of defense-related enzymes in apple and protects from fire blight. *European Journal of Plant Pathology* **106**: 529-536

Broome, J.C., English, J.T., Marois, J.J., Latorre, B.A., and Aviles, J.C. 1995. Development of an infection model for *Botrytis* bunch rot of grapes based on wetness duration and temperature. *Phytopathology* **85**: 97-102

Brown, W. 1922. On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and carbon dioxide. *Annals of Applied Botany* **36**: 257-283

Brown, J.E., Lu, T.Y., Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Collins, D.J., Wilson, M.A., Igwegbe, E.C.K., Chalutz, E., and Droby, S. 2001. The effect of low dose ultraviolet light-C seed treatment on induced resistance in cabbage to black rot. *Crop Protection* **20**: 873-883

Bulger, M.A., Ellis, M.A., and Madden, L.V. 1987. Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. *Phytopathology* **77**: 1225-1230

Buonaurio, R., Scarponi, L., Ferrara, M., Sidoti, P., and Bertona, A., 2002. Induction of systemic acquired resistance in pepper plants by acibenzolar-S-methyl against bacterial spot disease. *European Journal of Plant Pathology* **108**: 41-49

Burg, S.P., and Thimann, K.V. 1959. The physiology of ethylene formation in apples. *Proceedings of the National Academic for Science USA* **45**: 335-344

Burg, S.P., and Burg, E.A. 1965. Gas exchange in fruits. *Plant Physiology* **18**: 870-884

Burg, S.P. 1966. Fruit storage at sub-atmospheric pressures. *Science* **153**: 314-315

Calderon, A., Zapata, J.M., Munoz, R., Pedreno, M.A., and Barcelo, A.R. 1993. Resveratrol production as a part of the hypersensitive-like response of grapevine cells to an elicitor from *Trichoderma viride. New Physiologist* **124**: 455-463

Calderon, A., Zapata, J.M., and Barcelo, A.R. 1994. Peroxidase mediated formation of resveratrol oxidation products during the hypersensitive-like reaction of grapevine cells to an elicitor from *Trichoderma viride. Physiology and Plant molecular Biology* **44**: 289-299

Carver, T.L.W., Zeyen, R.J., Bushnell, W.R., and Robbins, M.P. 1994. Inhibition of phenylalanine ammonia lyase and cinnamyl alcohol dehydrogenase increases quantitative susceptibility of barley to powdery mildew (*Erysiphe graminis* D.C.). *Physiological and Molecular Plant Pathology* **44**: 261-272

Castoria, R., De Curtis, F., Lima, G., Caputo, L., Pacifico, S., and De Cicco, V. 2001. *Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogens of fruits: study on its modes of action. *Postharvest Biology and Technology* **22**: 7-17

Chalutz, E., and Stahmann, M. 1969. Induction of pisatin by ethylene. *Phytopathology* **59**: 1972-1973

Chappell, J., and Hahlbrock, K. 1984. Transcription of plant defence genes in response to UV light or fungal elicitor. *Nature* **311**: 76-78

Cheng, G.W., and Breen, P.J. 1991. Activity of phenylalanine ammonia lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *Journal of the American Society for Horticultural Science* **116(5)**: 865-869.

Chester, K. 1933. The problem of acquired physiological immunity in plants. *Quarterly Review of Biology* **8**: 129-154, 275-324

Clark, C.A., and Lorbeer, J.W. 1976. Comparative histopathology of *Botrytis cinerea* and *Botrytis squamosa* on onion leaves. *Phytopathology* **66**: 1279-1289

Cohen, Y., Gisi, U., and Niderman, T. 1993. Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology* **83** : 1054-1062

Cole, D.L. 1999. The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance, against bacterial and fungal diseases of tobacco. *Crop Protection* **18**: 267-273

Conconi, A., Smerdon, M.J., Howe, G.A., and Ryan, C.A. 1996. The octadecanoid signaling pathway in plants mediates a response to ultraviolet radiation. *Nature* **383**: 226-229

Constabel, C.P., and Ryan C.A. 1998. A survey of wound and methyl jasmonate induced leaf polyphenol oxidase in crop plants. *Phytochemistry* **47**: 507-511

Creelman, R.A., Tierney, M.L., and Mullet, J.E. 1992. Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulated wound gene expression. *Proceedings of the National Academy for Science U.S.A.* **89**: 4938-4941

Creelman R.A., and Mullet J.E. 1997. Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 355-381

Dann, E., Diers, B., Byrum, J., Hammerschmidt, R. 1998. Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies. *European Journal of Plant Pathology* **104**: 271-278

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negretto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., and Ward, E. 1994. A central role of salicylic acid in plant disease resistance. *Science* **266**: 1247-1249

Dhingra, O.D., and Sinclair, J.B. 1995. Basic plant pathology methods. Second edition. CRC Press, Lewis publishers

Dik, A.J., Koning, G., and Kohl, J. 1999. Evaluation of microbial antagonists for biological control of *Botrytis cinerea* stem infection in cucumber and tomato. *European Journal of Plant Pathology* **105**: 115-112

Dik, A.J. and Elad, Y.1999. Comparison of antagonists of *Botrytis cinerea* in greenhouse grown cucumber and tomato under different climatic conditions. *European Journal of Plant Pathology* **105**: 123-137

Dilley, D.R., and Carpenter, W.J. 1975. The role of chemical adjuvants and ethylene synthesis on cut flower longevity. *Acta Horticulturae* **41**: 117-132

Dilley, D.R. 1977. Hypobaric storage of perishable commodities-fruits, vegetables, flowers and seedlings. *Acta Horticulturae* **62**:61-70

Dixon, R.A., Lamb, C.J., and Harrison, M.J. 1994. Early events in the activation of plant defense responses. *Annual Review of Phytopathology* **32**: 479-501

Doares, S.H., Syrovers, T., Weiler, E., and Ryan C.A. 1995. Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proceedings of the National Academy of Science U.S.A.* **92**: 4095-4098

Dong, X. 1998. SA, JA, ethylene, and disease resistance in plants. *Current Opinions in Plant Biology* **1**: 316-323

Droby, S., Chalutz, E., Wilson, C.L. and Wisniewski, M.E. 1989. Characterization of the biocontrol activity of *Debaryomyces hansenii* in the control of *Penicilium digitatum* on grapefruit. *Canadian Journal of Microbiology* **35**: 794-800

Droby, S., Chalutz, E., Horev, B., Cohen, L., Gaba, V., Wilson, C.L., Wisniewski, M.E. 1993. Factors affecting UV-induced resistance in grapefruit against the green mould decay caused by *Penicillium digitatum. Plant Pathology* **42**: 418-425

Droby, S., Zahavi, T., Schena, L., Cohen, L., Wiess, B., Daus, A., Benarie, R., Chalutz, E., and Ippolito, A. 1998. Biocontrol of pre- and postharvest bunch decay of wine and table grapes. *Proceedings of the Seventh International Congress on Plant Protection,* Edinburgh, Scotland, 9-16 August, 5.2.67 (abstract).

Droby, S., Porat, R., Cohen, L., Weiss, B., Shapiro, S., Philisoph-Hadas, S., and Meir, S. 1999. Suppressing green mold decay in grapefruit with postharvest jasmonate application. *Journal of the American Society for Horticultural Science* **124** (2): 184-188

Dubery, I.A., Teodorczuk, L.G., and Louw A.E. 2000. Early responses in methyl jasmonate preconditioned cells toward pathogen derived elicitors. *Molecular Cell Biology Research Communication* **3**: 105-110

Ebel, J. 1986. Phytoalexin synthesis: the biochemical analysis of the induction process. *Annual Review of Phytopathology* **24**: 235-264

Ebel, J., and Cosio, E.G. 1994. Elicitors of plant defense responses. *International Review of Cytology* **148**: 1-36

Elad, Y. 1988. Latent infection of *Botrytis cinerea* in rose flowers and combined chemical and physiological control of the disease. *Crop Protection* 7: 361-366

Elad, Y. 1989. Effect of abiotic conditions on development of gray mold of rose and scanning electron microscopy. *Phytopathologia Mediterranea* **28**: 122-130

Elad, Y., Kirshner, B., and Gotlib, Y. 1993a. Attempts to control *Botrytis cinerea* on roses by pre- and postharvest treatments with biological and chemical agents. *Crop Protection* **12**: 69-73

Elad, Y., Kirshner, B., Gokkes, M., and Peer, R. 1993b. Disease symptoms caused by *Botrytis cinerea* in *Ruscus hypoglossum* plant and their control. *Phytoparasitica* **21(1)**: 53-61

Elad, Y., and Shtienberg, D. 1995. *Botrytis cinerea* in greenhouse vegetables: Chemical, cultural, physiological and biological controls and their integration. *Integrated Pest Management Review* **1**: 15-29

Elad, Y., and Evensen, K. 1995. Physiological aspects of resistance to *Botrytis cinerea*. *Phytopathology* **85**: 637-643

Elad, Y. 1997. Responses of plants to infection by *Botrytis cinerea* and novel means involved in reducing their susceptibility to infection. *Biological Reviews* **72**: 381-342

El-Gamassy, A.M. and El-Fattah, K.A. 1969. Pre expert treatments of rose cut flowers. *Annual Agricultural Science* (Cairo) **11**: 287-306

El-Ghaouth, A., Wilson, C.L., and Callahan, A.M. 2003. Induction of chitinse, β-1,3-glucanase and Phenylalanine ammonia lyase in peach fruit by UV-C treatment. *Phytopathology* **93**: 349-355

Elshof, P. 2000. The Dutch flower sector: structure trends and employment. www.ilo.org/public/english/dialoque/sectorpapers/flrsect/flrsect5.htm. 7/2/03

Ellis, M.B., and Walter, J.M. 1974. *Sclerotinia fuckeliana* (conidial state: *Botrytis cinerea*) In: 'CMI Descriptions of Pathogenic Fungi and Bacteria' No. 431, (Commonwealth Mycological Institute, Eastern Press Ltd.: London), pp. 2

Epple P., Apel, K., and Bohlmann, H. 1997. Overexpression of an endogenous thionin enhances resistance of *Arabidopsis* against *Fusarium oxysporum. The Plant Cell* **9**: 509-520

Epton, H.A., and Richmond, D.V. 1980. Formation, structure and germination of conidia. In: 'The Biology of *Botrytis*' (Eds. Coley-Smith, J.R., Verhoeff, K., and Jarvis, W.R.), pp. 41-84. Academic press, London, UK

Erb, K., Gallegly, M.E., and Leach, J.G. 1973. Longevity of mycelium of *Colletotrichum lindemuthianum* in hypocotyls tissue of resistant and susceptible bean cultivars. *Phytopathology* **63**; 1334-1335

Farhoomand, M.B., Kofranek, A.M.., Mor, Y., Reid, S.M., and Awad, A.R.W. 1980. Pulsing *Gladiolus hybrida* 'Captain Busch' with silver or quaternary ammonium compounds before low temperature storage. *Acta Horticulture* **109**: 253-258

Farmer, E.E., and Ryan, C.A. 1992. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *Plant Cell* **4**:129-134

Farnham, D.S., Barr, C., Marousky, M.J., Durkin, D., Rij, R., Thompson, J.F., Kofranek, A.M. 1979. Comparison of conditioning, pre-cooling, transit method, and use of a floral preservative on cut flower quality. *Journal of the American Society for Horticultural Science* **104**: 483-490

Field, A. 2000. Discovering statistics using SPSS for Windows. (Eds. Blakwell, G., de Leeuw, J., O'Muircheartaigh, C., Saris, W., Schuman, H., van Meter, K.), SAGE Publications, London, UK, pp. 275

Fischer, C.W. 1950. Ethylene gas, a problem in cut flower storage. *New York State Flower Growers Bulletin* **61**: 1,4

Flor, H.H. 1971. Current status of the gene-for-gene concept. *Annual Review of Phytopathology* **9**: 275-296

Fluhr, R. and Mattoo, A.K. 1996. Ethylene biosynthesis and perception. *Critical Reviews in Plant Sciences* **15**: 479-523

Friedrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Gut Rella, M., Meiers, B., Dincher, S., Staub, T., Uknes, S., Metraux, J-P., Kessmann, H., and Ryals, J. 1996. A Benzothiadiazole derivative induces systemic acquired resistance in tobacco. *Plant Journal* **10**: 61-70

Fritzemeier, K-H., Cretin, C., Kombrink, E., Rohwer, F., Taylor, J., Scheel, D., and Hahlbrock, K. 1987. Transient induction of phenylalanine ammonia-lyase and 4-coumarate:CoA legase mRNAs in potato leaves infected with virulent or avirulent races of *Phytophthora infestans. Plant Physiology* **85**: 34-41

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* **261**: 754-756

Garrod, B., Lewis, B.G., Brittain, M.J., and Davies, W.P. 1982. Studies on the contribution of lignin and suberin to the impedance of wounded carrot toot tissue to fungal invasion. *New Phytology* **90**: 99-108

Gast, K.L.B. 1997. Postharvest handling of fresh cut flowers and plant material. *Kansas State University, Agricultural Experiment Station and Cooperative Extension Service* pp. 7.

Glazebrook, J. 2001. Genes controlling expression of defense responses in *Arabidopsis. Current Opinion in Plant Biology* **2**: 280-286

Godard, J.-F., Ziadi, S., Monot, C., Le Corre, D., and Silue, D. 1999. Benzothiadiazole (BTH) induces resistance in cauliflower (*Brassica oleracea* var *botrytis*) to downy mildew of crucifers caused by *Peronospora parasitica. Crop Protection* **18**: 397-405

Gonzalez-Aguilar, G.A., Wang, C.Y., Buta, J.G., and Krizek, D.T. 2001. Use of UV-C irradiation to prevent decay and maintain postharvest quality of ripe 'Tommy Atkins' mangoes. *International Journal of Food Science and Technology* **36**: 767-773

Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.-H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., and Ryals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell* **8**: 629-643

Goszczynska, D.M., Rudnicki, R.M. 1988. Storage of cut flowers. *Horticultural Review* **10**: 35-62

Govrin, E.M., and Levine, A. 2002. Infection of *Arabidopsis* with a necrotrophic pathogen, *Botrytis cinerea,* elicits various defense responses but does not induce systemic acquired resistance (SAR). *Plant Molecular Biology* **48**: 267-276

Grisebach, H. 1981. Lignins. In 'The Biochemistry of Plants' (Ed. E.E. Conn), 7: 457-478. New York: Academic

Gundlach, H., Muller, M.J., Kutchan, T.M., and Zenk, M.H. 1992. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures, *Proceedings of the National Academy of Science U.S.A.* **89**: 2389-2393

Gupta, V., Willits, M.G., Glazebrook, J. 2000. *Arabidopsis thaliana EDS4* contributes to salicylic acid (SA)-dependent expression of defense responses: evidence for inhibition of jasmonic acid signaling by SA. *Molecular Plant Microbe Interactions* **13**: 503-5011

Hahlbrock, K., and Scheel, D. 1989. Physiology and molecular biology of phenylpropanoid pathway. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 347- 369

Halevy, A.H. and Mayak, S. 1974. Transport and conditioning of cut flowers. *Acta Horticulturae* **43**: 291-306

Halevy, A.H., Byrne, T.G., Kofranek, A.M., Farnham, D.S., Thompson, J.F., and Hardenburg, R.E. 1978. Evaluation of postharvest handling methods for transcontinental truck shipments of cut carnations, chrysanthemums and roses. *Journal of the American Society for Horticultural Science* **103**: 151-155

Halevy, A.H. and Mayak, S. 1981a. Senescence and postharvest physiology of cut flowers- Part 1. *Horticultural Review* : 204-236

Halevy, A.H. and Mayak, S. 1981b. Senescence and postharvest physiology of cut flowers- Part 2. *Horticultural Review* **3**: 59-143

Hammer, P.E., and Marois, J.J. 1988. Postharvest control of *Botrytis cinerea* on cut roses with Picro-cupric-ammonium formate. *Plant Disease* **72**: 347-350

Hammer, P.E., and Marois, J.J. 1989. Nonchemical methods for postharvest control of *Botrytis cinerea* on cut roses. *Phytopathology* **84**:1305-1312

Hammer, P.E., and Eversen, K.B. 1994. Differences between rose cultivars in susceptibility to infectio by *Botrytis cinerea. Phytopathology* **84**: 1305-1312

Hammerschmidt, R. 1999. Induced disease resistance: how do induced plants stop pathogens? *Physiological and Molecular Plant Pathology* **55**: 77-84

Hannusch, D.J. and Boland, G.J. 1996. Interactions of air temperature, relative humidity and biological control agents on grey mold of bean. *European Journal of Plant Pathology* **102**: 133-142

Hanson, A.D., and Kende, H. 1975. Ethylene-enhanced ion and sucrose efflux in morning glory flower tissue. *Plant Physiology* **55**: 663-669

Heale, J.B. 1992. Activation of host defence mechanisms in response to *Botrytis cinerea.* In: 'Recent advances of *Botrytis* Research' (Eds. Verhoeff, K., Malathrakis, M.E., and Williamson, B.), pp. 48-58. Pudoc Scientific Publishers, Wageningen, The Netherlands

Heldt, H-W. 1997. Plant biochemistry and molecular biology. Oxford University Press.pp. 347-350

Hennebert,G.L., and Gilles, G.L. 1958. Epidemiologie de *Botrytis cinerea* Pers. Sur les fraisiers. *Meded. Landbouwhogesch. Opzoekingsstn. Staat Gent* **23**: 864-888. In "*Botryotinia* and *Botrytis* species: taxonomy, physiology, and pathogenicity. A guide to the literature". (Ed. Jarvis, W.R.) 1977 Research Branch Canada Department of Agriculture, Monograph No 15

Herms, D.A., and Mattson, W.J. 1992. The dilemma of plants: to grow or defend. *The Quarterly Review of Biology* **67**: 283-335

Herve, J.J., and Moysan, J.P. 1967. Etudes de traitements diriges contre le Botrytis du fraisier (Botrytis cinerea). *Phytiatr. Phytopharm. Rev. FR. Med. Pharm. Veg.* 16 3-13. In "*Botryotinia* and *Botrytis* species: taxonomy, physiology, and pathogenicity. A guide to the literature". (Ed. Jarvis, W.R.) 1977 Research Branch Canada Department of Agriculture, Monograph No 15

Honda, Y., Toki, T., and Yunoki, T. 1977. Control of grey mould of greenhouse cucumber and tomato by inhibiting sporulation. *Plant Disease Reporter* **61**: 1041-1044

Huang, Y., Deverall, B.J., Tang, W.H., Wang, W., and Wu, FW 2000. Foliar application of acibenzolar-S-methyl and protection of postharvest rock melons and Hami melons from disease. *European Journal of Plant Pathology* **106**: 651-656

Il'inskaya, L.I., Goenburg, E.V., Chalenko, G.I., and Ozeretskovskaya, O.L. 1996. Involvement of jasmonic acid in the induction of potato resistance to *Phytophthora* infection. *Russian Journal of Plant Physiology* **43(5)**: 622-628

Ippolito, A., Nigro, F., Romanazzi, G., and Campanella, V. 1997. Field application of *Aureobasidium pullulans* against *Botrytis* storage rot of strawberries. *Proceedings of the COST* 914-915 'Non-conventional methods for the control of postharvest disease and microbiological spoilage' Bologna, Italy, October 9-11, 127-133

Ippolito, A., El Ghaouth, A., Wilson, C.L., and Wisniewski, M. 2000a. Control of postharvest decay of apple fruit *Aureobasidium pullulans* and induction of defence responses. *Postharvest Biology and Technology* **19**: 265-272

Ippolito, A. and Nigro, F.2000b. Impact of preharvest application of biological control agents on postarvest disease of fresh fruits and vegetables. *Crop Protection* **19**: 715-723

Isaac, S. 1992. *Fungal-Plant Interactions*. Chapman & Hall, London, New York, Tokyo, Melbourne, Madras.

Ishii, H., Tomita, Y., Horio, T., Narusaka, Y., Nakazawa, Y., Nishimura, K., and Iwamoto, S. 1999. Induced resistance of acibenzolar-S-methyl (CGA 245704) to cucumber and Japanese pear diseases. *European Journal of Plant Pathology* **105**: 77-85

Islam, S.Z., Honda, Y., and Sonhaji, M. 1998. Phototropism of conidial germ tubes of *Botrytis cinerea* and its implication in plant infection processes. *Plant Disease* **82**: 850-856

Jacobsen, B.J., and Backman, P.A. 1993. Biological and cultural plant disease controls: alternative and supplements to chemicals in IPM systems. *Plant Disease* **77**: 311-315

Janisiewicz, W.J., and Jeffers, S.N. 1997. Efficacy of commercial formulation of two biofungicides for control of blue mold and gray mold of apples in cold storage. *Crop Protection* **16**: 629-633

Jarvis, W.R. 1960. The apparatus for studying hygroscopic responses in fungal conidiophores. *Transactions of the British Mycological Society* **43**: 525-528

Jarvis, W.R. 1962a. The infection of strawberry and raspberry fruits by *Botrytis cinerea* Pers. *Annals of Applied Biology* **50**: 569-575

Jarvis, W.R. 1962b. The dispersal of spores of *Botrytis cinerea* in a raspberry plantation. *Transactions of the British Mycological Society* **45**: 549-559

Jarvis, W.R. 1964. The effect of some climatic factors on the incidence of grey mould of strawberry and raspberry fruit. *Horticultural Research* **3**: 65-71

Jarvis, W.R. 1977. *Botryotinia* and *Botrytis* species: taxonomy, physiology, and pathogenicity. A guide to the literature. Research Branch Canada Department of Agriculture, Monograph No 15

Jarvis, W.R. 1980a. Taxonomy. In 'The Biology of *Botrytis*' Eds. Coley-Smith, J.R., Verhoeff, K., and Jarvis, W.R. Academic Press, York, London pp. 3-17

Jarvis, W.R. 1980b. Epidemiology. In 'The Biology of *Botrytis*' Eds. Coley-Smith, J.R., Verhoeff, K., and Jarvis, W.R. Academic Press, York, London pp. 219-245

Joyce, D.C. 1988. Postharvest characteristics of Geraldton wax flowers. *Journal of the American Society for Horticultural Science* **13**: 738-742

Joyce, D.C. 1993. Postharvest floral organ fall in Geraldton waxflowers (*Chamelaucium uncinatum* Schauer). *Australian Journal of Experimental Agriculture* **33**:481-487

Joyce, D. C., and Patterson, B.P. 1993. Postharvest water relations in horticultural crops: Principles and Problems. In 'Postharvest Handling of Tropical Fruits', (Eds. B.R. Champ, E. Highley and G.I. Johnson), Proceedings No. 50, ACIAR pp. 228-238. Australian Centre for International Agricultural Research Canberra

Joyce, D.C., and Wearing, A. 1996. Fungicides fight flower drop. *Australian Horticulture* **94**: 58-59.

Joyce, D.C., and Johnson, G.I. 1999. Prospects for exploitation of natural disease resistance in harvested horticultural crops. *Postharvest News and Information* **10**: 45-48

Joyce, D.C., and Shorter, T.J. 2000. Long term, low temperature storage injures kangaroo paw cut flowers. *Postharvest Biology and Technology* **20**: 203-206

Kambil, A., and vanHeck, E. 1996. Competition in the Dutch flower markets. www.usc.edu/schools/bussines/atisp/ec/Dutch_flowers/flowercase.pdf. 9/2/03

Kamoen, O. 1972. Pathogenesis of *Botrytis cinerea* studies on tuberous begonia. Doctorale Thesis, *Fakulteit Landbouwwetenschappen, Rijksuniversiteit Gent* pp. 106

Kamoen, O., Jamart, G., Declereq, H., and Dubourdieu, D. 1980. Des eliciteurs de phytoalexines chez *Botrytis cinerea*. *Annals of Phytopathology* **12**: 365-376

Kamoen, O. 1992. *Botrytis cinerea*: host-pathogen interactions. In: "*Recent advances in Botrytis research*" (Eds. Verhoeff, K., Malathrakis, N.E., and Williamson, B.). Pudoc Scientific Publishers, Wageningen, 1992.

Καρατάγλης, Σ.Σ. 1999. *Φυσιολογία φυτών*. Τρίτη έκδοση. Εκδόσεις Art of text

Katz, V.A., Thulke, O.U., and Conrath, U. 1998. A Benzothiadiazole primes parsley cells for augmented elicitation of defense responses. *Plant Physiology* **117**: 1333-1339

Keen, N.T., and Yoshikawa, M. 1983. β-1,3-endoglucanase from soybean releases elicitor-active carbohydrates from fungus cell walls. *Plant Physiology* **71**: 460-465

Keller, H., Pamboukdjian, N., Ponchet, M., Poupet, A., Delon, R., Verrier, J-L., Roby, D., and Ricci, P. 1999. Pathogen-induced elicitin production in transgenic tobacco generates a hypersensitive response and nonspecific disease resistance. *The Plant Cell* **11**: 223-235

Kende, H., and Baumgartner, B. 1974. Regulation of aging in flowers of *Ipomoea tricolor* by ethylene. *Planta* **116**: 279-289

Kendra, D.F., and Hadwiger, L.A. 1984. Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum. Exp. Mycology* **8**: 276-282

Kerssies, A. 1993. Influence of environmental conditions on dispersal of *Botrytis cinerea* conidia and on postharvest infection of gerbera flowers grown under glass. *Plant Pathology* **42**: 754-762

Kerssies, A. 1994. Effects of temperature, vapor pressure deficit and radiation on infectivity of conidia of *Botrytis cinerea* and on susceptibility of gerbera petals. *European Journal of Plant Pathology* **100**: 123-136

Kerssies, A., Boskervanzessen, AI, and Frinking, H.D. 1995. Influence of environmental conditions in a glasshouse on conidia of *Botrytis cinerea* and on postharvest infection of rose flowers. *European Journal of Plant Pathology* **101**: 201-216

Kerssies, Aand Frinking, H.D. 1996. Relations between climate and dry weight of petals, epicuticular wax, cuticle, pre-harvest flowering period and susceptibility to *Botrytis cinerea* of gerbera and rose flowers. *European Journal of Plant Pathology* **102**: 257-263

Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., and Herzog, J. 1994. Induction of systemic acquired disease resistance in plants by chemicals. *Annual Review of Phytopathology* **32**: 439-459

Kessmann, H., Oostendorp, M., Ruess, W., Staub, T., Kunz, W., and Ryals, J. 1996. Systemic activated resistance – a new technology for plant disease control. *Pesticide Outlook* pp. 10-13.

Klarman, W.L., and Stanford, J.B. (1968) Isolation and purification of an antifungal principle from infected soybeans. *Life Sciences* **7**: 1095-1103

Kofranek, A.M. 1976. Opening flower buds after storage. *Acta Horticulture* **64**: 231-237

Kombrink, E., and Hahlbrock, K. 1986. Responses of cultured parsley cells to elicitors fro phytopathogenic fungi. Timing and dose dependency of elicitor-induced reactions. *Plant Physiology* **81**: 216-221

Kombrink, E., and Somssich, I. 1995. Defense responses of plants to pathogens. *Advances in Botanical Research* **21**: 2-34

Kombrink, E., and Schmelzer, E. 2001. The hypersensitive response and its role in local and systemic disease resistance. *European Journal of Plant Pathology* **107**: 69-78

Kuć, J. 1991. Phytoalexins: perspectives and prospects. In '*Mycotoxins and phytoalexins*' (Eds. Sharma, R., and Salunkhe, D.), Boca Raton: CRC. pp. 595-603

Kuć, J. 1995. Phytoalexins, stress metabolism and disease resistance in plants. *Annual Review of Phytopathology* **33**: 275-297

Kuć, J. 2000. Development and future direction of induced systemic resistance in plants. *Crop Protection* **19**: 859-861

Lancaster, D.M. 1975. Effects of saline water on keeping life of cut roses and carnations – a preliminary report. *Colorado Flower Growers Association Bulletin* **296**: 3-4

Last, F.T. 1960. Longevity of conidia of *Botrytis fabae Sardina. Transactions of the British Mycological Society* **43**: 673-680

Lawton, K.A., Potter, S.L., Uknes, S., and Ryals, J. 1994. Acquired resistance signal transduction in *Arabidopsis* is ethylene independed. *Plant Cell* **6**: 581-588

Lawton, K.A., Weymann, K., Friedrich, L., Vernooij, B., Uknes, S., and Ryals, J. 1995. Systemic acquired resistance in *Arabidopsis* requires salicylic acid but not ethylene. *Molecular Plant-Microbe Interactions* **8**: 863-870

Lawton, K., Friedrich, L., Hunt, M., Weymann, K., Delaney, T., Kessmann, H., Staub, T., and Ryals, J. 1996. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *Plant Journal* **10**: 71-82

Leach, C.M. 1962. The sporulation of diverse species of fungi under near ultraviolet light. *Canadian Journal of Botany* **40**, 1577-1602.

Leone, G. 1990. Significance and role of polygalacturonase production by *Botrytis cinerea* in pathogenesis. Ph.D. Thesis, Wageningen Agricultural University, The Netherlands pp. 80

Lima, G., Ippolito, A., Nigro, F., and Salerno, M. 1997. Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. *Postharvest Biology and Technology* **10**: 169-178

Lisker, N., Cohen, L., Chalutz, E., and Fuchs, Y. 1983. Fungal infections suppress ethylene–induced phenylalanine ammonia–lyase activity in grapefruits. *Physiological Plant Pathology* **22**: 331-338

Little, T.M. 1985. Analysis of percentage and rating scale data. *HortScience* **20**: 642-644

Liu, J., Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L. Adeyeye, O., Kabwe, M.K., Pusey, P.L., Chalutz, E., Sultana, T., and Droby, S. 1993. Application of ultraviolet-C light on storage rots and ripening of tomatoes. *Journal of Food Protection* **56**: 868-872

Liu, FW 1970. Storage of bananas in polyethylene bags with an ethylene absorbent. *HortScience* **5**: 25-27

Lucas, J.A. 1997. *Plant Pathology and Plant Pathogens.* 3rd edition, Blackwell Science Ltd. UK.

Lucas, J.A. 1999. Plant immunization: from myth to SAR. *Pesticide Science* **55**: 193-196

Lunz, J.M., and Hardenberg, R.E. 1968. The commercial storage of fruits, vegetables and florist and nursery stocks. *USDA Agricultural Handbook* **66**

Macnish, A.J., Joyce, D.C., Hofman, P.J., and Simons, D.H. 1999. Involvement of ethylene in postharvest senescence of *Boronia heterophylla* flowers. *Australian Journal of Experimental Agriculture* **39**: 911-913

Macnish, A.J., Joyce, D.C., Hofman, P.J., Simons, D.H., and Reid, M.S. 2000. 1-Mehtylcyclopropene treatment efficacy in preventing ethylene perception in banana fruit and grevillea and waxflower flowers. *Australian Journal of Experimental Agriculture* **40**: 471-481

MacRobbie, E.A. 1971. Fluxes and compartmentation in plant cells. *Annual Review of Plant Physiology* **22**: 75-90

Manslfield, J.W. 1980. Mechanisms of resistance to *Botrytis.* In: "The Biology of *Botrytis*" Eds. Coley-Smith, J.R., Verhoeff, K., and Jarvis, W.R. Academic Press, York, London p. 181-218

Marcus, L., and Schejter, A. 1983. Single step chromatographic purification and characterization of the endopolygalacturonases and pectinesterases of the fungus *Botrytis cinerea* Pers. *Physiological Plant Pathology* **23**: 1-13

Marois, J.J., Redmond, J.C., and MacDonald, J.D. 1988. Quantification of the impact of environment on the susceptibility of *Rosa hybrida* flowers to *Botrytis cinerea. Journal of the American Society for Horticultural Science* **113**: 842-845

Marquenie, D., Lammertyn, J., Geeraerd, A.H., Soontjens, C., Van Impe, J.F., Nicolai, B.M., and Michiels, C.W. 2001. Inactivation of conidia of *Botrytis cinerea* and *Monilinia fructigena* using UV-C and heat treatment. *International Journal of Food Microbiology* in press

Marquenie, D., Lammertyn, J., Geeraerd, A.H., Soontjens, C., Van Impe, J.F., Nicolai, B.M., and Michiels, C.W. 2002. Using survival analysis to investigate the effect of UV-C and heat treatment on storage rot of strawberry and sweet cherry. *International Jurnal o Food Microbiology* **73**: 187-196

Martinez-Tellez, M.A., and Lafuente, M.T. 1993. Chilling-induced changes in phenylalanine ammonia-lyase, peroxidase, and polyphenol oxidase activities in citrus flavedo tissue. *Acta Horticulturae* **343**: 257-265

Matern, U., and Kneusel, R. 1988. Phenolic compounds in plant disease resistance. *Phytoparasitica* **16**: 153-170

Mauch, F., Hadwiger, L.A., and Boller, T. 1988. Antifungal hydrolases in pea tissue. 2. Inhibition of fungal growth by combinations of chitinase and β-1,3-glucanase in pea pods by pathogens and elicitors. *Plant Physiology* **76**: 607-611

Mauch-Mani, B., and Slusarenko, A.J. 1996. Production of salicylic acid precursors is a major function of phenylalanine ammonia lyase in the resistance of *Arabidopsis* to *Peronospora parasitica. The Plant Cell* **8**: 203-212

Maxie, E.C., Farnham, D.C., Mitchell, F.G., Sommer, N.F., Parsons, R.A. Snyder, R.G., and Rae, H.L. 1973. Temperature and ethylene effects on cut flowers of carnation (*Dianthus caryophyllus* L.). *Journal of American Society for Horticultural Science* **98**: 568-572

Mayak, S., Bravdo, B., Gvulli, A., and Halevy, A.H. 1973. Improvement of opening of cut gladioli flowers by pretreatment with high sugar concentration. *Scientia Horticulture* **1**: 357-365

Mayak, S., and Kofranek, A. 1976. Altering the sensitivity of carnation flowers (*Dianthus caryophyllus* L.) to ethylene. *Journal of the American Society for Horticultural Science* **101**: 503-506

McClellan, W.D., and Hewitt, W.B. 1973. Early *Botrytis* rot of grapes: time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. *Phytopathology* **63**: 1151-1156

Meir, S., Droby, S., Davidson, H., Alsvia, S., Cohen, L., Horev, B., and Philosoph-Hadas, S. 1998. Suppression of Botrytis rot in cut rose flowers by postharvest application of methyl jasmonate. *Postharvest Biology and Technology* **13**: 235-243

Meneses, J.F., and Mondeiro, A.A. 1990. Permanent ventilation in non heated greenhouses to reduce *Botrytis* on tomatoes, *Proceedings from the International Seminar of British-Israel Workshop on Greenhouse Technology,* Tel-Aviv, Israel

Métraux, J-P., Ahl Goy, P., Staub, T., Speich, J., Steinmann, A., Ryals, J., and Ward, E. 1991. Induced systemic resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens. In: 'Advances in Molecular Genetics of Plant-Microbe Interactions', (Eds. Hennecke, H., and Verma, D.P.S.), pp. 432-439, Kluver Academic Publishers, Dordrecht

Métraux, J-P. 2001. Systemic acquired resistance and salicylic acid: current state of knowledge. *European Journal of Plant Pathology* **107**: 13-18

Mitter, N., Kazan, K., Way, H. M., Broekaert, W.F., and Manners, J.M. 1998. Systemic induction of an *Arabidopsis* plant defensin gene promoter by tobacco mosaic virus and jasmonic acid in transgenic tobacco. *Plant Science* **136**: 169-180

Moline, H., Hubbard, J. E., Karns, K.S., Buyer, S., and Cohen, D. 1999. Selective isolation of bacterial antagonists of *Botrytis cinerea.European Journal of Plant Pathology* **105**: 95-101

Muller, R., Lind-Iversen, S., Stummann, B.M., Serek, M. 2000. Expression of genes for ethylene biosynthetic enzymes and an ethylene receptor in senescing flowers of miniature roses. *Journal for Horticultural Science and Biotechnology* **75**: 12-18

Nair, N.G., and Allen, R.N. 1993. Infection of grape flowers and berries by *Botrytis cinerea* as a function of time and temperature. *Mycological Research* **97**:1012-1014

Narusaka, Y., Narusaka,M., Horio, T., and Ishii, H. 1999. Induction of disease resistance in cucumber by acibenzolar-S-methyl and expression of resistance-related genes. *Annals of the Phytopathological Society of Japan* **65**: 116-122

Nasser, W., De Tapia, M., Kauffmann, S., Montasser-Kouhsari, S., and Burkard, G. 1988. Identification and characterization of maize pathogenesis related proteins. Four maize PR proteins are chitinases. *Plant Molecular Biology* **11**: 529-538

Nelson, K.E. 1991. The grape. In 'Quality and Preservation of Fruits' (Ed. Eskin, N.M.M.), CRC Press, Boston, USA, pp. 125-165

Nichols, R. 1966. Ethylene production during senescence of flowers. *Journal of Horticultural Science* **41**: 279-290

Nichols, R. 1967. Investigations on the cold storage of cut flowers. *Proceedings 12th International Congress, Madrid* **3**: 329-337

Nichols, R. 1971. Refrigeration and storage of cut flowers. *Refrigeration and Air Condition* **74**: 36-39

Nichols, R. 1977. A descriptive model of the senescence of the carnation (*Dianthus caryophyllus*) inflorescence. *Acta Horticulturae* **71**: 227-232

Nichols, R., and Kofranek, A.M. 1982. Reversal of ethylene inhibition of tulip stem elongation by silver thiosulphate. *Scientia Horticulture* **17**: 71-79

Nigro, F., Ippolito, A., Lima, G. 1998. Use of UV-C light to reduce *Botrytis* storage rot of table grapes. *Postharvest Biology and Technology* **13**: 171-181

Niki, T., Mitsuhara, I., Seo, S., Ohtsubo, N., and Ohashi, Y. 1998. Antagonistic effect of salicylic acid and jasmonic acid on the expression o pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. *Plant Cell Physiology* **39(5)**: 500-507

Nojiri, H., Sugimori, M., Yamane, H., Nishimura, Y., Yamada, A., Shibuya, N., Kodama, O., Murofushi, N., and Omori, T. 1996. Involvement of jasmonic acid in elicitor-induced phytoalexin production in suspension-cultured rice cells. *Plant Physiology* **110**:387-392

Nowak, J., and Rudnicki, R.M. 1979. Long term storage of cut flowers. *Acta Horticulturae* **91**: 123-134

Nurnberger, T., Nennstiel, D., Jabs, T., Sacks, W.R., Hahlbrock, K., and Scheel, D. 1994. High affinity binding of a fungal oligopeptide elicitor to parsley plasma membranes triggers multiple defenses responses. *The Plant Cell* **78**: 449-460

Nurnberger, T., and Scheel, D. 2001. Signal transmission in the plant immune response. *Trends in Plant Science* **6(8)**: 372-379

O'Donnell, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O., and Bowles, D.J. 1996. Ethylene as a signal mediating the wound response of tomato plants. *Science* **274**: 1914-1917

Ogawa, J.M., Gilpatric, J.D., and Ciarapa, L. 1977. Review of plant pathogens resistance to fungicides and bactericides. In ' FAO Plant Protection Bulletin, A Publication of he World Reporting Service on Plant Diseases and Pests' **25**: pp. 97-110.

O'Neil, T.M., Niv., A., Elad, Y., and Shtienberg, D. 1996. Biological control of *Botrytis cinerea* on tomato stem wounds with *Trichoderma harzianum. European Journal of Plant Pathology* **102**: 635-543

Pady, S.M., and Kelly, C.D. 1954. Aerobiological studies of fungi and bacteria over the Atlantic Ocean. *Canadian Journal of Botany* **32**: 202-212

Pady, S.M., and Kapica, L. 1956. Fungi in air masses over Montreal during 1950-1951. *Canadian Journal of Botany* **34**: 1-15

Pallas, J.A., Paiva, N.L., Lamb, C., and Dixon, R.,A. 1996. Tobacco plants epigenetically suppressed in phenylalanine ammonia lyase expression do not develop systemic acquired resistance in response to infection by tobacco mosaic virus. *The Plant Journal* **10**: 281-293

Panagiotarou, N. and Chrisaugi, M. 1998. Manual of chemical control of plant diseases. *Benaki Phytopathological Institute,* Kifissia, pp. 204.

Park, T.K., and Polacco, J.C. 1989. Distinct lipoxygenase species appear in the hypocotyls/radicle of germinating soybean. *Plant Physiology* **90**: 285-290

Parthier, B. 1991. Jasmonates, new regulators of plant growth and development: many facts and few hypotheses on their actions. *Botanica Acta* 446-454

Paull, R.E., and Goo, T. 1982. Pulse treatments with silver nitrate extends vase life of anthuriums. *Journal of the American Society for Horticultural Science* **107**: 842-844

Pearce, G., Strydom, D., Johnson, S., and Ryan, C.A. 1991. A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science* **253**: 895-898

Pena-Cortes, H., Albrecht, T., Prat, S., Weiler, E.W., and Willmitzer, L. 1993. Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. *Planta* **191**: 123-128

Penninckx, I.A.M.A., Eggermont, K., Terras, F.R.G., Thomma, B.P.H.J., De Samblanx, G.W., Buchala, A., Metraux, J-P., Manners J.M., and Broekaert, W.F. 1996. Pathogen-induced systemic activation of a plant defensin gene in *Arabidopsis* follows a salicylic acid-independed pathway. *The Plant Cell* **8**: 2309-2323

Perrin, D.R., and Cruickshank, I.A.M. 1965. Studies on phytoalexins VII. The chemical stimulation of pisatin formation in *Pisum sativum* L. *Austrian Journal of Biological Science* **18**: 803-816

Pie, K. and de Leeuw, G.T.N. 1991. Histopathology of the initial stages of interaction between rose flowers and *Botrytis cinerea*. *Netherlands Journal of Plant Pathology* **97**: 335-344

Pieterse, C.M.J., van Wees, S., Hoffland, E. van Pelt, J.A., van Loon, L.C. 1996. Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of

salicylic acid accumulation and pathogenesis related gene expression. *Plant Cell* **8**:1225-1237

Pieterse, C.M.J., van Wees, S., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J., and van Loon L.C. 1998. A nobel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *The Plant Cell* **10**: 1571-1580

Pieterse, C.M.J., and van Loon, L.C. 1999. Salicylic acid-independent plant defence pathways. *Trends in Plant Science* **4(2)**: 52-58

Powelson, R.L. 1960. Initiation of strawberry fruit rot caused by *Botrytis cinerea*. *Phytopathology* **50**: 491-494

Purvis, M.J., Collier, D.C., and Walls, D. 1966. Laboratory Techniques in botany, second edition, London, Butterworths

Rao, M.V., Lee, H-I., Creelman, R.A., Mullet, J.E., Davis, K.R. 2000. Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. *The Plant Cell* **12**: 1633-1646

Raskin, I. 1992. Role of salicylic acid in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**: 439-463

Reid, M.S., and Kofranek, S.T. 1980. Postharvest physiology of cut flowers. *Chron. Horticulture* **20**: 25-27

Reid, M.S., Paul, J.L., Farhoomand, M.B., Kofranek, A.M., and Staby, G.L. 1980. Pulse treatments with the silverthiosulfate complex extend the vase life of cut carnations. *Journal of the American Society for Horticultural Science* **105**: 25-27

Redolfi, P. 1983. Occurrence of pathogenesis related and similar proteins in different plant species. *Netherlands Journal of Plant Pathology* **89**: 245-254

Reglinski, T., Poole, P.R., Whitaker, G., and Hoyte, S.M. 1997. Induced resistance against *Sclerotinia sclerotiorum* in kiwifruit leaves. *Plant Pathology* **46**: 716-721

Ride, J.P., and Pearce, R.B. 1979. Lignification and papilla formation at sites of attempted penetration of wheat leaves by non-pathogenic fungi. *Physiological Plant Pathology* **15**: 79-92

Rijkenberg, F.H.J., de Leeuw, G.T.N., and Verhoeff, K. 1980. Light an electron microscopy studies on the infection of tomato fruit by *Botrytis cinerea. Canadian Journal of Botany* **58**: 1394-1404

Rippel, A., and Heilman, F. 1930. Quantitative Undersuchungen uber die Wirkung der Kohlensaure auf Heterotrophen. *Arch. Mikrobiol* **1**: 119-136. In '*Botryotinia* and *Botrytis* species: taxonomy, physiology, and pathogenicity. A guide to the literature'. (Eds. Jarvis, W.R), 1977 Research Branch Canada Department of Agriculture, Monograph No 15

Rogers, M.N. 1962. Sell flowers that last. (Reprints of 9 papers). *Floricultural Review* 130-131.

Rogers, M.N. 1963. Living flowers that last - a national symposium. (Ed. Rogers, M.N.) University of Missouri Press, Columbia.

Rogers, M.N. 1973. An historical and critical review of postharvest physiology research on cut flowers. *HortScience* **8**:189-194

Rojo, E., Titarenko, E., Leon, J., Berger, S., Vancanneyt, G., and Sanchez-Serrano, J.J. 1998. Reversible protein phosphorylation regulates jasmonic acid-dependent and independent wound signal transduction pathways in *Arabidopsis thaliana. The Plant Journal* **13**: 153-165

Rojo, E., Leon, J., and Sanchez-Serrano, J.J. 1999. Cross-talk between wound signaling pathways determines local versus systemic gene expression in *Arabidopsis thaliana. The Plant Journal* **20**: 136-142

Ross, A.F. 1961. Systemic acquired resistance induced by localized virus infections in plants. *Virology* **14**: 3410-358

Ruess, W., Mueller, K., Knauf-Beiter, G., Kenz, W., and Staub, T. 1996. Plant activator CGA 245704: an innovative approach for disease control in cereals and tobacco. *Brighton Crop Protection Conference-Pests and Diseases* **2A-6,** pp. 53-60, 18-21 November 1996

Ruzin, S.E. 1999. Plant Microtechnique and Microscopy. Oxford University Press, pp. 93-95

Ryals, J.A., Ward, E., and Metraux J.P. 1992. Systemic acquired resistance: An inducible defense mechanism in plants. In 'Inducible Plant Proteins: Their Biochemistry and Molecular Biology', (Ed. J.L. Wray), Cambridge UK: Cambridge University Press pp. 205-229

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H-Y., and Hunt, M.D. 1996. Systemic Acquired Resistance. *The Plant Cell* **8**: 1809-1819

Salinas, J., Glandorf, D.C.M., Picavet, F.D., and Verhoeff, K. 1989. Effects of temperature, relative humidity and age of conidia on the incidence of specking on gerbera flowers caused by *Botrytis cinerea. Netherlands Journal of Plant Pathology* **95**: 51-64

Salinas, J. 1990. Protection of gerbera flowers against infection of *Botrytis cinerea* with anticutinase monoclonal antibodies. *Acta Botanica Neerlandia* (abstract) **39**: 313-314

Salinas, J. 1992. Function of cutinolitic enzymes in the infection process of gerbera flowers by *Botrytis cinerea*. Ph.D. Thesis, University of Utrecht, The Netherlands, pp. 105

Salinas, J., and Verhoeff, K. 1995. Microscopic studies of the infection of gerbera flowers by *Botrytis cinerea*. *European Journal of Plant Pathology* **101**: 377-386

Salinger, J.P. 1985. Commercial flower growing. Wellington, N.Z.: Butterworths Horticultural. Freesia, pp. 121-131

Schaller, A., and Ryan, C.A. 1995. Systemin – a polypeptide defense signal in plants. *BioEssays* **18(1)**: 27-33

Schena, L., Ippolito, A., Zahavi, T., Cohen, L., Nigro, F., and Droby, S. 1999. Genetic diversity and biocontrol activity of *Aureobasidium pullulans* isolates against postharvest rots. *Postharvest Biology and Technology* **17**: 189-199

Schroder, M., Hahlbrock, K., and Kombrink, E. 1992. Temporal and spatial patterns of 1,3-β-glucanase and chitinase induction in potato leaves infected by *Phytophthora infestans*. *Plant Journal* **2**: 161-172

Sembdner, G., and Parthier, B. 1993. The biochemistry and the physiological and molecular actions of jasmonates. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**: 569-589

Serek, M., Sisler, E.C., and Reid, M.S. 1994. Novel gaseous ethylene binding inhibitor prevents ethylene effects in potted flowering plants. *Journal of the American Society for Horticultural Science* **119**: 1230-1233

Serek, M., Sisler, E.C. 2001. Efficacy of inhibitors of ethylene binding in improvement of the postharvest characteristics of potted flowering plants. *Postharvest Biology and Technology* **23**: 161-166

Sharkey, T.D. 1996. Emission of low molecular mass hydrocarbons from plants. *Trends in Plant Science* **3**: 78-82

Sharp, J., Albersheim, P., Ossowski, O., Pilotti, A., Garegg, P., and Lindberg, B. 1984. Comparison of the structure of elicitor activities of a synthetic and mycelial-wall-derived hexa-(β-D-glucopyranosyl)-D-glusitol. *Journal of Biological Chemistry* 259: 11341-11345

Shoemaker, P.B., and Lorbeer, J.W. 1977. The role of dew and temperature in the epidemiology of *Botrytis* leaf blight of onion. *Phytopathology* **61**: 910

Shtienberg, D., Elad, Y., Niv, A., Nitzani, Y., and Kirshner, B. 1998. Significance of leaf infection by *Botrytis cinerea* in stem rotting of tomatoes grown in non-heated greenhouses. *European Journal of Plant Pathology* **104**: 753-763

Shulaev, V., Silverman, P., and Raskin, I. 1997. Airborne signaling by methyl salicylate in plant pathogen resistance. *Nature* **385**: 718-721

Smock, R.M. 1944. The elimination from the atmosphere of ethylene evolved by apples. *Proceedings of the American Society for Horticultural Science* **44**:134-140

Sommer, N.F. 1985. Principles of disease suppression by handling practices. In: *Postharvest Technology of Horticultural Crops*. (University of California, Division of Agriculture and Natural Resources) pp.75-83

Sreeramula, T. 1959. The diurnal and seasonal periodicity of spores of certain plant pathogens in the air. *Tranactions of the British Mycological Society* **42**: 177-184

Stack, J.P. 1998. Postharvest biological control: commercial successes and a model for public and private sector cooperation. CPP98, *7th International Congress of Plant Pathology, 'Invited papers-abstracts'* (Vol.2), Edinburgh, UK pp. 5.2.2.S.

Staswick, P.E. 1990. Novel regulation of vegetative storage protein genes. *Plant Cell* **2**: 1-6

Staub, T., Ahl-Goy, P., and Kessmann, H. 1992. Chemically induced disease resistance in plants. In 'Proceedings of the 10th international Symposium of Systemic Fungicides and Antifungal Compounds' (Eds. Lyr, H., and Polter, C.), pp. 239-249

Stermer, B.A. 1995. Molecular regulation of systemic induced resistance. In "Induced Resistance to Disease in Plants". Eds. R. Hammerscmidt and Kuć, J. Kluwer Academic Publishers pp. 111-140

Stevens, C., Wilson, C.L., Lu, J.Y., Khan, V.A., Chalutz, E., Droby, S., Kabwe, M.K., Haung, Z., Adeyeye, O., Pusey, L.P., Wisniewski, M.E., and West, M. 1996. Plant hormesis induced by ultraviolet light-C for controlling postharvest disease of tree fruits. *Crop Protection* **15**: 129-134

Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Pusey, P.L., Kabwe, M.K., Igwegbe, E.C.K., Chalutz, E., and Droby, S. 1998. The germicidal and hormetic effects of UV-C light on reducing brown rot disease and yeast microflora of peaches. *Crop Protection* **17**: 75-83

Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Chalutz, E., Droby, S., Kabwe, M.K., Haung, Z., Adeyeye, O., Pusey, L.P., and Tang, A.Y.A. 1999. Induced resistance of sweetpotato to *Fusarium* root rot by UV-C hormesis. *Crop Protection* **18**: 463-470

Sticher, L., Mauch-Mani, B., and Metraux, J.P. 1997. Systemic Acquired Resistance. *Annual Review of Phytopathology* **35**: 235-270

Strider, D.L. 1985. Diseases of Floral Crops. Vol 2. Praeger Publishers, New York.

Suo, Y., and Leung, D.W.M. 2001. Elevation of extracellular β-1,3-glucanase and chitinase in rose in response to treatment with acibenzolar-S-methyl and infection by *D. rosae*. *Journal of Plant Physiology* **158**: 971-976

Suttle, J.C., and Kende, H. 1978. Ethylene and senescence in petals of *Tradescantia*. *Plant Physiology* **62**: 267-271

Suttle, J.C., and Kende, H. 1980. Ethylene action and loss of membrane integrity during petal senescence in *Tradescantia*. *Plant Physiology* **65**: 1065-1072

Sutton, J.C., James, T.D.W., and Rowell, P.M. 1986. BOTCAST: a forecasting system to time the initial fucgicide spray for managing Botrytis leaf blight of onions. *Agricultural Ecosystem and Environment* **18**: 123-143

Swinburne, T.R. 1983. Quiescent infection in postharvest diseases. In: 'Post-harvest Pathology of Fruits and Vegetables' (Ed Dennis, C), Academic Press, London, pp. 1-21

Sytsema-Kalkman, E.C., Brijn-Jansen, L.L., and Marissen, N. 1995. Carbohydrate status of cut freesia flowers. *Acta Horticulturae* **405**, *VI International Symposium on Postharvest Physiology of Ornamental Plants* (abstract)

Szandala, E.S., and Backhouse, D. 2001. Suppression of sporulation of *Botrytis cinerea* by antagonists applied after infection. *Australasian Plant Pathology* **30**: 165-170.

Taiz, L. and Zeiger, E. 1998. Plant Physiology, 2nd ed. Sinauer Associates, Inc., Publishers, pp. 61-80.

Tan, K.K., and Epton, H.A.S. 1973. Effects of light on the growth and sporulation of *Botrytis cinerea*. *Transactions of the British Mycological Society* **61**: 145-157

Tan, K.K., and Epton, H.A.S. 1974. Further studies on light and sporulation in *Botrytis cinerea*. *Transactions of the British Mycological Society* **62**: 105-112

Taylor, M.N., Joyce, D.C., Wearing, A.H., and Simons, D.H. 1997. Influence of fungal pathogens and environmental conditions on disease severity, flowers fall and desiccation

of harvested Geraldton waxflower 1. Studies with model packages. *Australian Journal of Experimental Agriculture* **37**: 817-824

Taylor, M.N., Joyce, D.C., Wearing, A.H., and Simons, D.H. 1999. Evaluation of pyrimethanil (Scala) for the control of *Botrytis cinerea* on harvested Geraldton waxflower. *Australian Journal of Experimental Agriculture* **39**: 639-641

Terras, F.R.G., Eggermont, K., Kovaleve, V., Raikhel, N.V., Osborn, R.W., Kester, A., Reesm S.B., Torrekens, S., Van Leuven, F., Vanderleyden, J., Cammue, B.P.A., and Broekaert, W.F. 1995. Small cysteine-rich antifungal proteins from radish: their role in host defense. *Plant Cell* **7**: 573-588

Terry, L.A., and Joyce, D.C. 2000. Suppression of grey mould on strawberry fruit with the chemical plant activator acibenzolar. *Pest Management Science* **56**: 989-992

Terry, L.A. 2002. Natural disease resistance in strawberry fruit and Geraldton waxflower flowers. PhD Thesis, Cranfield University

Thaller, J.S., Stout, M.J., Karban, R., and Duffey, S.S. 1996. Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. *Journal of Chemical Ecology* **22**: 1767-1781

Thaller, J.S., Fidantsef, A.L., Duffey, S.S., and Bostock R.M. 1999. Trade-offs in plant defense against pathogens and herbivores: a field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology* **25**: 1597-1609

Thomma, B. P.H.J., Eggermont, K., Penninckx, I.A.M.A., Mauch-Mani, B., Vogelsang, R., Cammue, B.P.A., and Broekaert, W. 1998. Separate jasmonate-dependent and salicylate-dependent defense response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proceedings of the National Academy of Science, USA* **95**: 15107-15111

Thomma, B. P.H.J. Eggermont, K., Broekaert, W., and Cammue, B.P.A. 2000. Disease development of several fungi on *Arabidopsis* can be reduced by treatment with methyl jasmonate. *Plant Physiology and Biochemistry* **38**: 421-427

Thomma, B. P.H.J., Penninckx, I.A.M.A., Broekaert, W.F., and Cammue, B.P.A. 2001. The complexity of disease signaling in *Arabidopsis*. *Current Opinion in Immunology* **13**: 63-68

Titarenko, E., Rojo, E., Leon, J., and Sanchez-Serrano, J.J. 1997. Jasmonic acid-dependent and independent signaling pathways control wound-induced gene activation in *Arabidopsis thaliana*. *Plant Physiology* **115**: 817-826

Tomas, A., Wearing, A.H., and Joyce, D.C. 1995. *Botrytis cinerea*: a causal agent of premature flower drop in packaged Geraldton waxflower. *Australasian Plant Pathology* **24**: 26-28

Tosi, L., Zazzerini, A. 1999. Benzothiadiazole induces resistance to *Plasmopara helianthi* in sunflower plants. *Journal of Phytopathology* **147**: 365-370

Uknes, S., Mauch-Mani, B., Moyer, M., P., Potter, S., Williams, S., Dincher, D., Chandler, A., Slusarenko, E., Ward, E., and Ryals, J. 1992. Acquired resistance in *Arabidopsis*. *Plant Cell* **4**: 645-656

Vakalounakis, D.J. 1991. Control of early blight of greenhouse tomato, caused by *Alternaria solani*, by inhibiting sporulation with ultraviolet-absorbing vinyl film. *Plant Disease* **76**: 43-46

Van der Molen, G.E., Labavich J.H., Strand, J.J., and DeVay, J.E. 1983. Pathogen-induced vascular gels: Ethylene as a host intermediate. *Plant Physiology* **59**: 573-580

Van der Sman, R.G.M., Evelo, R.G., Wilkinson, W.G., van Doorn. 1996. Quality loss in packed rose flowers due to *Botrytis cinerea* infection as related to temperature regimes and packaging design. *Postharvest Biology and Technology* 7: 341-350

Van Doorn, W.G., and Perik, R.R.J. 1990. Hydroxyquinoline citrate and low pH prevent vascular blockage in stems of cut rose flowers y reducing the number of bacteria. *Journal of the American Society for Horticultural Science* **115**: 979-981

Van Loon, L.C., and Gerritsen, Y.A.M. 1989. Localization of pathogenesis-related proteins in infected and non-infected leaves of Samsun NN tobacco during the hypersensitive reaction to tobacco mosaic virus. *Plant Science* **63**: 131-140

Van Loon, L.C., Pierpoint, W.S., Boller, T., and Conejero, V. 1994. Recommendations for naming plant pathogenesis-related proteins. *Plant Molecular Biology Reporter* **12**: 245-264

Van Loon, L.C. 1997. Induced resistance in plants and the role of pathogenesis-related proteins. *European Journal of Plant Pathology* **103**: 753-765

Van Meeteren, U., and van Gelder, H., and van de Peppel, A.C. 1995 Aspects of carbohydrate balance during floret opening in freesia. *Acta Horticulturae* **405,** *VI International Symposium on Postharvest Physiology of Ornamental Plants* (abstract)

Van Toor, R.F., Jaspers, M.V., and Stewart, A. 2001. Evaluation of acibenzolar-S-methyl for induction of resistance in camellia flowers to *Ciborinia camelliae. New Zealand Plant Protection* **54**: 209-212

Veen, H. 1979. Effects of silver on ethylene synthesis and action in cut carnations. *Planta* **145**: 467-470

Veen, H. 1983. Silver thiosulphate: An experimental tool in plant science. *Scientia Horticulture* **181**: 155-160

Verhoeff, K. 1980. The infection process and host pathogen interactions. In: 'The Biology of *Botrytis*' Eds. Coley-Smith, J.R., Verhoeff, K., Jarvis, W.R., pp.153-180, Academic Press, London, UK

Vernooij, B., Friedrich, L., Morse, A., Reist, R., Kolditz-Jawhar, R., Ward, E., Uknes, S., Kessmann, H., and Ryals, J. 1994. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *Plant Cell* **6**: 959-965

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahi-Goy, P., Metraux, J.P., and Ryals, J.A. 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* **3**: 1085-1094

Wasternack, C., and Parthier, B. 1997. Jasmonate-signalled plant gene expression. *Trends in Plant Science* **2**: 302-307

Whinspear, KW., Postlethwaite, J.D., and Cotton R.F. 1970. The restriction of *Cladosporium fulvum* and *Botrytis cinerea* attacking glasshouse tomatoes by automatic humidity control. *Annals of Applied Biology* **65**: 75-83

White, R.F. 1979. Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology* **99**: 410-412

Williamson, B., Duncan, G.H., Harrison, J.G., Harding, L.A., Elad, Y., and Zimand, G. 1995. Effect of humidity on infection of rose petals by dry-inoculated conidia of *Botrytis cinerea*. *Mycological Research* **99**: 1303-1310

Willingham, S.L., Pegg, K.G., Langdon, P.W.B., Cooke, A.W., Peasley, D., and Mclennan. 2002. Combinations of strobilurin fungicides and acibenzolar (Bion) to reduce scab on passionfruit by *Cladosporium oxysporum*. *Australasian Plant Pathology* **31**: 333-336

Wilson, A.R. 1937. The chocolate spot disease of beans caused by *Botrytis cinerea* Pers. *Annals of Applied Biology* **24**: 258-288

Wilson, C.L., and El- Chaouth, A. 1993. Multifaceted biological control of postharvest disease of fruits and vegetables. In 'Pest management: Biologically based technologies' (Eds. Lumsden, R.D. and Vaughn, J.L.), American Chemical Society, Washington, D.C.

Wilson, M. 1997. Biocontrol of aerial plant diseases in agriculture and horticulture: current approaches and future prospects. *Journal of Industrial Microbiology and Biotechnology* **19**: 188-191

Wilson, C.L., Chaouth, A.El., Upchurch, B., Stevens, C., Khan, V., Droby, S., and Chalutz, E. 1997. Using an on-line UV-C apparatus to treat harvested fruit for controlling postharvest decay. *HortTechology* **7**: 278282

Winkler, A.J., Cook, J.A., Kliewer, W.M., and Lider, L.A. 1974. Pre-cooling, Fumigation and Storage of Table Grapes. In 'General Viticulture', University of California Press, California, USA, pp. 593-621

Xu, Y., Linda Chang, P-F., Liu, D., Narasimhan, M.L., Raghothama, K.G., Hasegawa, P.M., and Bressan, R.A. 1994. Plant defense genes are synergistically induced by ethylene and methyl jasmonate. *The Plant Cell* **6**: 1077-1085

Yang, S.F. 1980. Regulation of ethylene biosynthesis. *HortScience* **15**: 238-243

Yoshikawa, M., Yamaoka, N., and Takeuchi, Y. 1993. Elicitors: their significance and primary modes of action in the induction of plant defense reactions. *Plant Cell Physiology* **34**: 1163-1173

Zainuri, Joyce, D.C., Wearing, A.H., Coates, L., and Terry, L.A. 2001. Effects of phosphonate and salicylic acid treatments on anthracnose disease development and

ripening of 'Kensington Pride' mango fruit. *Australian Journal of Experimental Agriculture* **41**: 805-813

Ziadi, S., Barbedette, S., Godard, J.F., Monot, C., Le Corre, D. and Silue, D. 2001. Production of pathogenesis-related proteins in the cauliflower (*Brassica oleracea* var. *botrytis*)-downy mildew (*Peronospora parasitica*) pathosystem treated with acibenzolar-S-methyl. *Plant Pathology* **50**: 579-586

Zimand, G., Elad, Y., and Chet, I. 1996. Effect of *Trichoderma harzianum* on *B. cinerea* pathogenicity. *Phytopathology* **86**: 1255-1260

Zucker, M. 1965. Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. *Plant Physiology* **40**: 779-784.

APPENDIX 1: GENERAL INTRODUCTION

Table A1.1: Share of retail spots as a percent (%) of total sales in the main EU countries in 1999.

Country	Florists	Supermarkets	Market/street vendors	Garden centres	Others	Total
Italy	80	2	15	2	1	100
France	58	16	9	8	9	100
Germany	64	10	8	-	18	100
The Netherlands	48	18	26	3	5	100
UK	35	41	9	4	11	100

Source: Estimates from different publications (Horticulture Commodity Board, 1999, The Netherlans).

Table A1.2: Top 25 flower species sold at The Netherlands auctions in 1998-2000 in thousand units (single stems). Source: The Federation of Netherlands Flower Auctions, 2001.

Product	Year		
	1998	1999	2000
Rosa	3,031,359	3,220,103	3,200,668
Dendranthema	1,389,105	1,426,455	1,437,945
Tulip	1,123,174	1,199,430	1,131,352
Gerbera	544,365	626,086	658,934
Dianthus	652,899	678,117	572,987
Freesia	484,244	452,452	443,693
Lilium	342,306	354,094	380,779
Astromeria	267,853	281,358	305,232
Hypericum	141,435	139,189	198,748
Narcissus	161,189	183,138	196,026
Gypsophila	170,620	190,084	193,272
Iris	175,838	201,894	180,719
Solidago	163,586	175,376	169,909
Eustoma	106,836	121,708	116,275
Limonium	84,139	90,230	92,445
Hippeastrum	87,552	84,183	90,097
Gladiolus	72,808	62,762	77,361
Helianthus	81,111	76,464	71,569
Aster	72,567	75,780	68,693
Waxflower	54,981	74,499	66,209
Anemone	59,981	53,473	61,357
Asparagus	59,568	54,639	57,445
Anthurium	45,977	49,031	54,892
Veronica	44,953	51,845	51,896
Tanacetum	50,065	48,097	49,632

APPENDIX 2: GENERAL MATERIALS AND METHODS

A2.1. MEDIA AND BUFFERS

A2.1.1 Half strength PDA

Potato Dextrose Agar (OXOID Ltd. Basingstoke, Hampshire, UK) 19.5g

Distilled water 1L

The media was sterilised in an autoclave at 123°C for 30 min.

A2.1.2 Buffers and reagents

A2.1.2.1 Sodium borate buffer pH 8.8

75% Sodium borate (1 M boric acid and 1 M NaOH)

25% Hydrochloric acid (0.1 M HCl)

A2.1.2.2 Potassium phosphate buffer pH 7.2

71.7% 1 M K_2HPO_4

28.3% 1 M KH_2PO_4

A2.1.2.3 Folin-Ciocalteu reagent

One mL of the sample was added to 65 mL deionized water. Contents were swirled to mix. Five mL Folin-Ciocalteu reagent were added and mixed again. After 1 min and before 8 min 15 mL of 20% sodium carbonate solution were added and mixed again

A2.1.2.4 Lactophenol aniline-blue

Lactophenol (100 mL)

20 mL phenol

20 mL Lactic acid

40 mL Glycerin

20 mL Distilled water

Aniline blue solution

6 mL glacial acetic acid

3 mL 1 % aqueous aniline blue

Lactophenol was mixed with aniline blue.

A2.2 PLANT MATERIAL

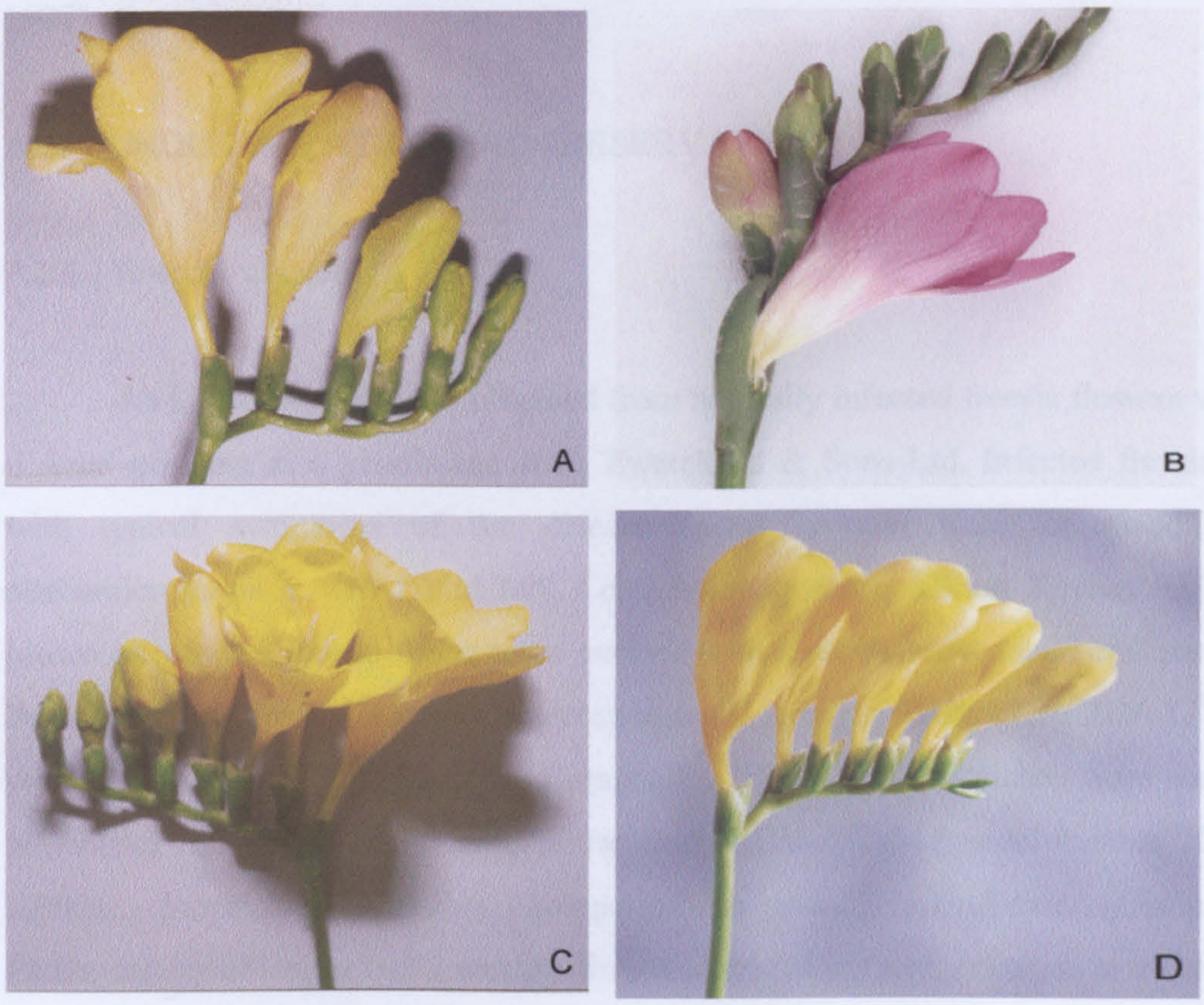

Plate A2.1: Freesia flowers vars. 'Cinderella' (A), Cote d'Azur' (B), 'Dukaat' (C) and 'Texel' (D) used for experiments.

Plate A2.2: Freesia var. 'Cote d'Azur' at the maturity stage used in experiments (Gast, 1997).

A2.3 FUNGUS ISOLATION AND PRESERVATION

A2.3.1 Botrytis cinerea

An isolate was initially obtained from naturally infected freesia flowers var. 'Cote d'Azur' growing in a glasshouse at C. Zwetsloots & Sons Ltd. Infected freesia flowers with typical symptoms of the disease (petal lesions) were examined under a stereomicroscope at x10 (Wild M5. Leica Microsystems, Milton Keynes, UK). Small pieces of infected freesia tissue were excised with a sterile razor blade and put on 9 cm Petri dishes containing ½ potato dextrose agar (½ strength PDA; 19.5g PDA L^{-1} distilled water) (OXOID Ltd. Basingstoke, Hampshire, UK). The Petri dishes were sealed with Nescofilm® and incubated at 20°C under diurnal UV-A light (model number 10531, GE lighting, Surrey, UK) (12h) photoperiod to induce sporulation (Leach, 1962). Pathogenicity of isolate BcF1 was confirmed in preliminary experiments to affirm Koch's postulates. A single-spore culture from the isolate was produced from a *B. cinerea* conidium picked with a sterile biological needle under the stereomicroscope. The spore was placed on the surface of ½ strength PDA on a Petri dish and incubated at 20°C for 12 days until the isolate produced adequate spore numbers. Mycelium plugs from 12-14 day old cultures were long-term (e.g. 6 months) stored in sterile distilled water at 4°C

(Bosewinkel, 1976). The fungus was revitalised regularly by culturing it through sterilised fresh tomato pieces placed on ½ PDA. Conidiophores and conidia of the fungus were taken from tomato tissue with a sterile razor blade and placed aseptically on ½ strength PDA plates (Dhingra and Sinclair, 1995).

Prior to flower inoculation BcF1 was sub-cultured on ½ strength PDA for 12-14 days at 20°C under diurnal (12h) photoperiod UV-A light. After 12-14 days the fungus produced sufficient numbers of spores for inoculation.

A2.4 PREFORMED ANTIFUNGAL COMPOUNDS IN FREESIA FLOWERS

A2.4.1 Freesia flower crude extraction

Frozen flowers in -18°C were lyophilised, ground with a mortar and pestle and subjected to sequential extraction in ethyl acetate, chloroform-methanol (1:1 v/v) and 80% ethanol at 6 mL per gram fresh weight (Adikaram *et al.*, 2002) using an Ultra-Turrax T25 Homogeniser at 9500 rpm for 3 minutes for each extractant. The extract was filtered through Whatman No. 3 filter paper under vacuum after each extraction. The filtrate was evaporated to dryness in a rotary evaporator at 35°C. The residue was stored at –18°C for future use.

A2.4.2 Thin layer chromatography

Glass-backed thin layer chromatography (TLC) plates (20 x 10 cm) coated with silica gel 60 or 60 F_{254} (Merck, Darmstadt, Germany) were used. The plates were spotted (5 – 10 µl) with re-suspended crude freesia flower extracts using 5 or 10 µl micro-pipettes. Extracting solvents (99% v/v) were used as positive controls. TLC plates were developed in one dimension at room temperature (approximately 22°C) in a TLC tank (20 x 20 x 10 cm) lined with filter paper (i.e. solvent saturated atmosphere). One-dimensional TLCs were developed in running solvents of hexane: ethyl acetate: methanol (A = 60:40:1; B = 60:40:10; C = 60: 40: 20; D = 60:40:30 v/v/v; 100 mL) (Zainuri *et al.*, 2001; Terry, 2002).

A2.4.3 Antifungal activity bioassays

After 24h air drying, the plates were inoculated by spraying with either a *Cladosporium cladosporioides* (provided by Dr Leon Terry) or the pathogen *B. cinerea* (BcF1) conidial suspensions (10^6 spores mL^{-1}) in Czapek - Dox nutrient solution (Klarman and Stanford, 1968). The plates were placed in sealed plastic transparent boxes. Therein, TLC plates were placed on top of a plastic tray above wet paper tissue to maintain 100% RH. The boxes were kept at 22°C and incubated for 2-3 days until fungal mycelium growth was visible.

A2.5 DISEASE ASSESSMENTS


B


C


D

Plate A2.3. Disease severity scores: 0: no spots on the petals (A), 1 = 1-5% petal coverage by spots (B), 2 = 5-25% petal coverage by spots (C), 3 = 25-50% petal coverage by spots (D) and 4 = 50-100% petal coverage by spots (E).

APPENDIX 3: FACTORS POTENTIALLY AFFECTING INFECTION OF FREESIA FLOWERS BY *B. CINEREA*

APPENDIX 3.1: CUT FREESIA FLOWER REJECTIONS IN RELATION TO PRE-HARVEST ENVIRONMENTAL CONDITIONS

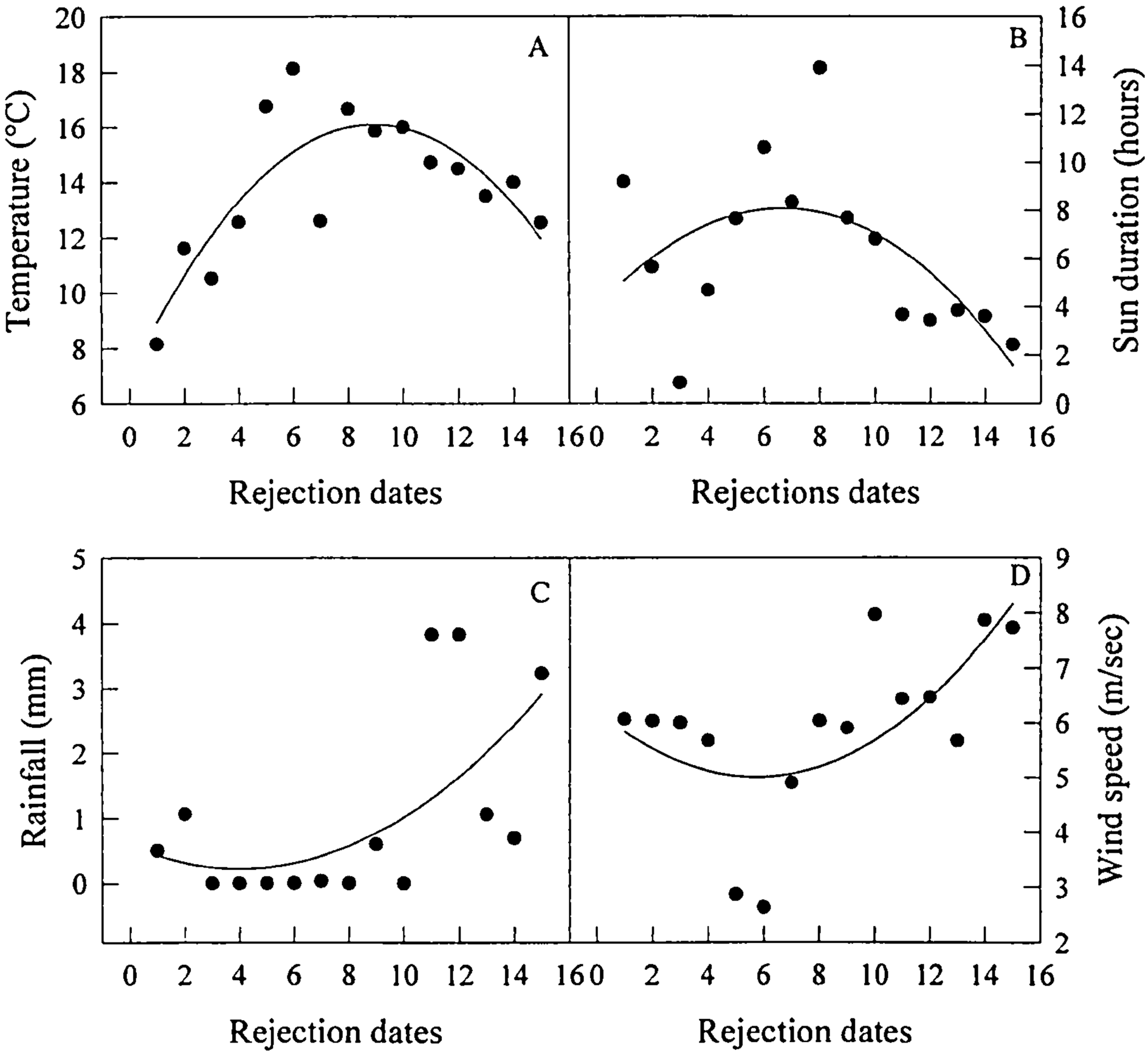

Figure A3.1.3: Rejection dates (total of 15) in year 2000 of freesia stems in the UK as a function of temperature (A), sun duration (B) rainfall (C) and wind speed (D) in the region of Vlissingen, Holland. Y-axis data are 3-day-means preceding harvest. Correlations: A: $y = 7.03 + 2.02x - 0.11x^2$, $R^2 = 0.67$, $P = 0.001$. B: $y = 3.89 + 1.25x - 0.09x^2$, $R^2 = 0.32$, $P = 0.1$. C: $y = 0.59 - 0.18x + 0.023x^2$, $R^2 = 0.38$, $P = 0.06$. D: $y = 6.22 - 0.42 + 0.04x^2$, $R^2 = 0.4$, $P = 0.05$.

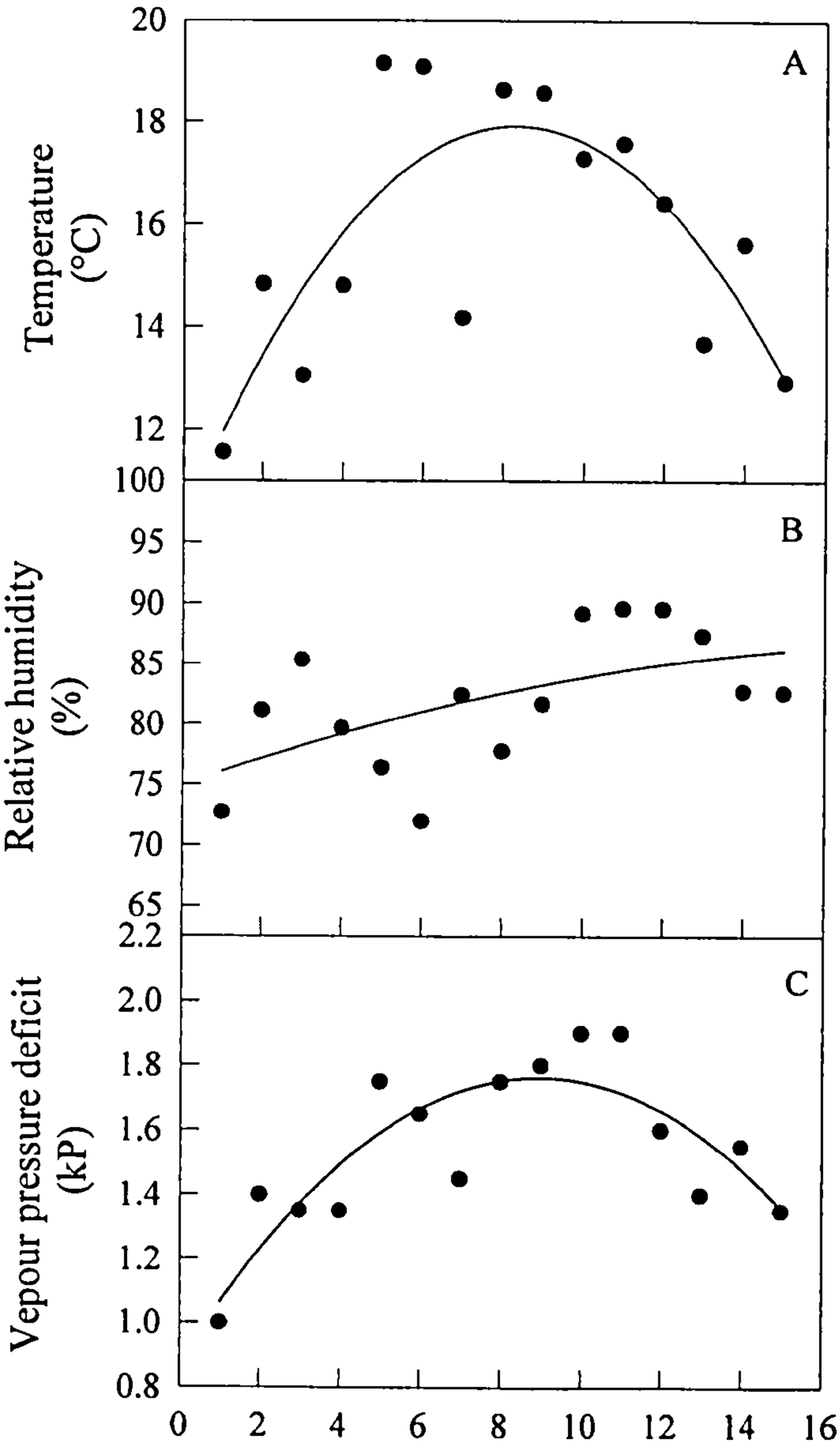

Figure A3.1.4: Rejection dates (total of 15) in year 2000 of freesia stems in the UK as a function of temperature (A), relative humidity (RH) (B) and vapour pressure deficit (VPD) (C) inside the glasshouse in Holland. Y-axis data are 3-day-means preceding harvest. Correlations: A: $y = 10.23 + 1.23x - 0.11x^2$, $R^2 = 0.6$, $P = 0.004$. B: $y = 74.88 + 1.2x - 0.03x^2$, $R^2 = 0.33$, $P = 0.09$. C: $y = -14 + 21.25x - 6.97x^2$, $R^2 = 0.11$, $P = 0.61$.

APPENDIX 3.2: EFFECTS OF TEMPERATURE, RELATIVE HUMIDITY AND INOCULUM LEVEL ON POSTHARVEST INFECTION OF FREESIA FLOWERS BY *BOTRYTIS CINEREA* - MICROSCOPICAL STUDIES

Table A3.2.1: Non-parametric analysis (Kruskal-Wallis test) for disease severity rating scale of freesia var. 'Cote d'Azur' flowers inoculated with 10^2, 10^3, and 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C under 80-90% RH or 100% RH.

Inoculum	N		Mean rank
10^2	180		249.24
10^3	180		252.32
10^4	180		309.94
	540		
Variable	df	Chi square	Asymp. Sig.
Disease severity	2	18.777	.000

Table A3.2.2: Non-parametric analysis (Kruskal-Wallis test) for disease severity rating scale of freesia var. 'Cote d'Azur' flowers inoculated with 10^2, 10^3, and 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C under 80-90% RH or 100% RH.

Temperature (°C)	N		Mean rank
5	180		229.70
12	180		309.33
20	180		272.47
	540		
Variable	df	Chi square	Asymp. Sig.
Disease severity	2	18.777	.000

Table A3.2.3: Non-parametric analysis (Kruskal-Wallis test) for disease severity rating scale of freesia var. 'Cote d'Azur' flowers inoculated with 10^2, 10^3, and 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C under 80-90% RH or 100% RH.

RH (%)	N		Mean rank
80-90	270		157.76
100	270		383.24
	540		
Variable	df	Chi square	Asymp. Sig.
Disease severity	1	306.216	.000

Table A3.2.4: ANOVA table for disease severity of freesia var. 'Cote d'Azur' flowers inoculated with 10^2, 10^3, and 10^4 *B. cinerea* conidia mL^{-1}, incubated at 5, 12, and 20°C under 100% RH (sleeved) or 80-90% RH (unsleeved).

Source	Sum Squares	ofdf	Mean Square	F	Sig.
Corrected Model	500.898	17	29.465	62.412	0.000
Intercept	544.409	1	544.409	1153.178	0.000
INOCULUM (I)	52.002	2	26.001	55.076	0.000
TEMPERATURE (T)	23.133	2	11.567	24.500	0.000
SLEEVE (S)	355.591	1	355.591	753.220	0.000
I * T	5.315	4	1.329	2.815	0.025
I * S	33.871	2	16.936	35.873	0.000
T * S	24.456	2	12.228	25.902	0.000
I * T * S	6.529	4	1.632	3.457	0.008
Error	246.433	522	.472		
Total	1291.740	540			
Corrected Total	747.331	539			

Table A3.2.5 Corresponding estimated parameters (i.e. disease severity and inoculum level) for the linear model ($y = y_0 + ax$) used in Figure 3.8 to describe the effects of temperature and RH on the disease severity on freesia flower petals. Flowers were initially incubated for 24h at 5°C then transferred at 5°C for 48h and 72h.

Temperature (°C)	**RH (%)**	**Time (hours)**	**Estimated parameters**		**Coefficient**	**Significance**
			yo	**a**	**R^2**	**P**
5	80-90	24	0	0	Inf [a]	-
5	100		0.55	-0.12	0.99	0.04
12	80-90		-0.63	0.36	0.79	0.30
12	100		0.17	0.40	0.46	0.53
20	80-90		0	0	Inf	-
20	100		-0.22	0.74	0.93	0.18
5	80-90	48	0	0	Inf	-
5	100		0.33	0.41	0.52	0.47
5	80-90		-0.10	0.19	0.81	0.29
5	100		0.65	0.34	0.20	0.70
5	80-90		0	0	Inf	-
5	100		0.02	0.64	0.81	0.29
5	80-90	72	0	0	Inf	-
5	100		0.65	0.54	0.90	0.20
5	80-90		-0.1	0.19	0.81	0.29
5	100		0.75	0.43	0.45	0.53

5	80-90	0	0	Inf	-
5	100	0.53	0.68	0.83	0.27

[a] Inf: Infinite

Table A3.2.6: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers inoculated with 10^2, 10^3, and 10^4 *B. cinerea* conidia mL^{-1}, incubated at 5, 12, and 20°C under 100% RH (sleeved) or 80-90% RH (unsleeved).

Source	Sum Squares	ofdf	Mean Square	F	Sig.
Corrected Model	469822.876	17	27636.640	69.460	0.000
Intercept	469286.224	1	469286.224	1179.475	0.000
INOCULUM (I)	60022.493	2	30011.246	75.428	0.000
TEMPERATURE (T)	26011.493	2	13005.746	32.688	0.000
SLEEVE (S)	304213.869	1	304213.869	764.592	0.000
I * T	11754.874	4	2938.719	7.386	0.000
I * S	38863.226	2	19431.613	48.838	0.000
T * S	21120.804	2	10560.402	26.542	0.000
I * T * S	7836.119	4	1959.030	4.924	0.001
Error	207691.900	522	397.877		
Total	1146801.00	540			
Corrected Total	677514.776	539			

Table A3.2.7: Corresponding estimated parameters parameters (i.e. disease severity and inoculum level) for the linear model ($y = y_0 + ax$) used in Figure 3.9 to describe the effects of temperature and RH on the lesion number on freesia flower petals. Flowers were initially incubated for 24h at 5°C then transferred at 5°C for 48h and 72h.

Temperature (°C)	**RH (%)**	**Time (hours)**	**Estimated parameters**		**Coefficient**	**Significance**
			yo	**a**	**R^2**	**P**
5	80-90	24	0	0	Inf [a]	-
5	100		0	0	Inf	-
12	80-90		-11.88	7.79	0.95	0.14
12	100		-0.37	14.29	0.49	0.5
20	80-90		0	0	Inf	-
20	100		2.23	16.8	0.57	0.46
5	80-90	48	3.52	-0.41	0.62	0.42
5	100		16.25	8.71	0.40	0.56
5	80-90		-7.13	7.80	0.99	0.6
5	100		10.11	12.51	0.25	0.67
5	80-90		0	0	Inf	-
5	100		-2.02	20.67	0.65	0.37
5	80-90	72	3.52	-0.41	0.62	0.42
5	100		13.06	16.05	0.89	0.21
5	80-90		-6.00	8.00	1.00	<0.001
5	100		24.21	9.92	0.15	0.74
5	80-90		0	0	Inf	-
5	100		3.23	21.65	0.66	0.39

[a] Inf: Infinite

Table A3.2.8: ANOVA table for lesion diameter of freesia var. 'Cote d'Azur' flowers inoculated with 10^2, 10^3, and 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C under 100% RH (sleeved).

Source	Sum Squares	ofdf	Mean Square	F	Sig.
Corrected Model	3.741	8	.468	8.046	0.000
Intercept	131.881	1	131.881	2269.371	0.000
INOCULUM (I)	.252	2	.126	2.170	0.117
TEMPERATURE (T)	2.203	2	1.102	18.958	0.000
I * T	1.348	4	.337	5.798	0.000
Error	13.424	231	5.811E-02		
Total	147.555	240			
Corrected Total	17.165	239			

Table A3.2.9: Corresponding estimated parameters parameters (i.e. disease severity and inoculum level) for the linear model ($y = y_0 + ax$) used in Figure 3.10 to describe the effects of temperature and RH on the lesion diameter on freesia flower petals. Flowers were initially incubated for 24h at 5°C then transferred at 5°C for 48h and 72h.

Temperature (°C)	**RH (%)**	**Time (hours)**	**Estimated parameters**		**Coefficient**	**Significance**
			yo	**a**	**R^2**	**P**
5	100	24	0	0	Inf [a]	-
12			0.74	-0.09	0.90	0.20
20			0.53	0.05	0.85	0.25
5	100	48	0.64	0.02	0.03	0.89
5			0.81	-0.05	0.57	0.47
5			0.51	0.07	0.52	0.43
5	100	72	1.12	-0.02	0.20	0.91
5			1.37	-0.18	0.80	0.30
5			0.87	-0.03	0.52	0.43

[a] Inf: Infinite

APPENDIX 4: NOVEL STRATEGIES TO CONTROL POSTHARVEST SPECKING OF CUT FREESIA FLOWERS CAUSED BY *B. CINEREA*

APPENDIX 4.1: SUPPRESSION USING POSTHARVEST TREATMENT WITH ACIBENZOLAR-S-METHYL

A4.1.1 Effect of acibenzolar-S-methyl on artificially inoculated flowers (experiment A1)

Table A4.1.1.1: Non-parametric test (Kruskal-Wallis) for disease severity rating scale of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C.

Treatments	N		Mean Rank
Control	40		96.29
Acibenzolar 1	40		75.14
Acibenzolar 2	40		78.82
Acibenzolar 3	40		71.75
Total	160		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	3	6.760	0.080

Table A4.1.1.2: Non-parametric test (Kruskal-Wallis) for disease severity rating scale of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C.

Temperature (°C)	N		Mean Rank
5	60		41.65
12	60		113.45
20	40		89.35
Total	160		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	75.110	.000

Table A4.1.1.3: ANOVA table for disease severity of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	70.183	11	6.380	15.043	.000
Intercept	333.396	1	333.396	786.036	.000
CHEMICAL TREATMENT (C)	5.227	3	1.742	4.108	.008
TEMPERATURE (T)	61.121	2	30.561	72.052	.000
C * T	3.627	6	.604	1.425	.209
Error	62.774	148	.424		
Total	471.390	160			
Corrected Total	132.957	159			

Table A4.1.1.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05.

Treatments [a]	N	1	2	3	4	5
Acibenzolar 1 5 [b]	15	.5867				
Acibenzolar 2 5	15	.6333				
Acibenzolar 3 5	15	.7133	.7133			
Control 5	15	.8667	.8667			
Acibenzolar 3 20	10		1.1900	1.1900		
Acibenzolar 1 20	10			1.5300	1.5300	
Control 20	10				1.8000	
Acibenzolar 2 20	10				1.8600	
Acibenzolar 2 12	15				1.8933	
Acibenzolar 3 12	15				1.9400	
Acibenzolar 1 12	15				1.9867	
Control 12	15					2.6400
Sig.		.327	.079	.186	.123	1.000

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AI L^{-1}, respectively. Control = 0 g AI L^{-1}

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.1.1.5: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	145121.000	11	13192.818	26.556	.000
Intercept	663609.643	1	663609.643	1335.801	.000
CHEMICAL TREATMENT (C)	11171.414	3	3723.805	7.496	.000
TEMPERATURE (T)	129731.333	2	64865.667	130.570	.000
C * T	5026.717	6	837.786	1.686	.128
Error	73524.600	148	496.788		
Total	875030.000	160			
Corrected Total	218645.600	159			

Table A4.1.1.6: Lesion number means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4	5	6
Acibenzolar 3 5 [b]	15	21.3333					
Acibenzolar 1 5	15	24.4667					
Acibenzolar 2 5	15	31.6667					
Control 5	15	34.0667					
Acibenzolar 3 20	10		58.1000				
Acibenzolar 1 20	10			75.9000			
Acibenzolar 1 12	15			78.4000	78.4000		
Control 20	10			79.4000	79.4000	79.4000	
Acibenzolar 3 12	15			85.5333	85.5333	85.5333	85.5333
Acibenzolar 2 12	15				96.1333	96.1333	96.1333
Acibenzolar 2 20	10					98.0000	98.0000
Control 12	15						104.0000
Sig.		.190	1.000	.325	.065	.052	.054

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AI L^{-1}, respectively. Control = 0 g AI L^{-1}

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.1.1.7: ANOVA table for lesion diameter of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.225	11	.202	7.236	.000
Intercept	91.569	1	91.569	3275.512	.000
CHEMICAL TREATMENT (C)	1.178	3	.393	14.044	.000
TEMPERATURE (T)	.681	2	.340	12.173	.000
C * T	.268	6	4.472E-02	1.600	.152
Error	3.578	128	2.796E-02		
Total	102.648	140			
Corrected Total	5.804	139			

Table A4.1.1.8: Lesion diameter means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatment [a]	N	1	2	3
Acibenzolar 2 20 [b]	10	.6340		
Acibenzolar 1 20	10	.7120	.7120	
Acibenzolar 3 20	10	.7620	.7620	
Acibenzolar 2 5	10	.7780	.7780	
Control 20	10	.7790	.7790	
Acibenzolar 1 5	10	.7840	.7840	
Acibenzolar 2 12	15		.8113	
Acibenzolar 3 12	15		.8147	
Acibenzolar 3 5	10		.8170	
Acibenzolar 1 12	15		.8253	
Control 12	15			1.0707
Control 5	10			1.0950
Sig.		.062	.183	.730

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AI L^{-1}, respectively. Control = 0 g AI L^{-1}

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.1.1.9: Parameters estimated for the linear model ($y = y_0 + ax + bx^2$) used to describe the effects of acibenzolar concentration and incubation temperature on disease severity, lesion number and lesion diameter on freesia petals.

Temperature (°C)	Estimated parameters			Coefficient (R^2)
	y_0	a	b	
a. Disease severity				
5	0.84	-1.57	2.26	0.82
12	2.56	-3.82	4.54	0.61
20	1.70	0.79	-2.63	0.61
b. Lesion number				
5	31.79	-11.30	-9.09	0.49
12	99.15	-66.48	78.78	0.26
20	74.66	123.12	-247.47	71.4
c. Lesion diameter				
5	1.08	– 1.72	2.12	0.89
12	1.33	– 2.92	3.43	0.78
20	0.79	– 0.89	1.39	0.93

Table A4.1.1.10: One-way ANOVA table for lesion diameter of detached petals. Freesia var. 'Cote d'Azur' flowers were treated with four acibenzolar concentrations.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.102	3	1.367	.908	.447
Within Groups	54.203	36	1.506		
Total	58.305	39			

A4.1.2 Effect of acibenzolar-S-methyl on un-inoculated (naturally infected) flowers (experiment A2)

Table A4.1.2.1: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates, left un-inoculated (naturally infected) and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.225	11	.202	7.236	.000
Intercept	91.569	1	91.569	3275.512	.000
CHEMICAL TREATMENT (C)	1.178	3	.393	14.044	.000
TEMPERATURE (T)	.681	2	.340	12.173	.000
C * T	.268	6	4.472E-02	1.600	.152
Error	3.578	128	2.796E-02		
Total	102.648	140			
Corrected Total	5.804	139			

A4.1.3 Effect of acibenzolar-S-methyl on artificially inoculated flowers (experiment A3)

Table A4.1.3.1: Non-parametric test (Kruskal-Wallis) for disease severity rating scale of freesia var. 'Texel' flowers treated with acibenzolar-S-methyl, iprodione and acibenzolar + iprodione. Flowers were artificially inoculated with 0, 10^3, 10^4, and 10^5 *B. cinerea* conidia L^{-1} and incubated at 5, 12 and 20°C.

Treatments	N		Mean Rank
Control	240		533.99
Acibenzolar	240		463.82
Iprodione	240		476.55
Acibenzolar + Iprodione	240		447.65
Total	960		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	3	14.424	.002

Table A4.1.3.2: Non-parametric test (Kruskal-Wallis) for disease severity rating scale of freesia var. 'Texel' flowers treated with acibenzolar-S-methyl, iprodione and acibenzolar + iprodione. Flowers were artificially inoculated with 0, 10^3, 10^4, and 10^5 *B. cinerea* conidia L^{-1} and incubated at 5, 12 and 20°C.

Temperature	N	Mean Rank
5	384	438.10
12	384	502.36
20	192	521.59
Total	960	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	17.004	.000

Table A4.1.3.3: Non-parametric test (Kruskal-Wallis) for disease severity rating scale of freesia var. 'Texel' flowers treated with acibenzolar-S-methyl, iprodione and acibenzolar + iprodione. Flowers were artificially inoculated with 0, 10^3, 10^4, and 10^5 *B. cinerea* conidia L^{-1} and incubated at 5, 12 and 20°C.

Temperature	N	Mean Rank
0	240	211.85
10^3	240	347.00
10^4	240	572.29
10^5	240	790.85
Total	960	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	3	663.360	.000

Table A4.1.3.4: ANOVA table for disease severity of freesia var. 'Texel' flowers treated with water, acibenzolar-S-methyl, iprodione and acibenzolar + iprodione inoculated with 0, 10^3, 10^4 and 10^5 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C.

Source	Sum Squares	ofdf	Mean Square	F	Sig.
Corrected Model	1886.754	47	40.144	65.314	.000
Intercept	2088.334	1	2088.334	3397.715	.000
CHEMICAL TREATM (C)	25.174	3	8.391	13.653	.000
TEMPERATURE (T)	28.929	2	14.464	23.533	.000
INOCULUM (I)	1605.648	3	535.216	870.795	.000
C * T	13.911	6	2.318	3.772	.001
C * I	15.146	9	1.683	2.738	.004
T * I	11.423	6	1.904	3.098	.005
C * T * I	17.095	18	.950	1.545	.068
Error	560.542	912	.615		
Total	4686.000	960			
Corrected Total	2447.296	959			

Table A4.1.3.5: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Texel' flowers treated with water, acibenzolar-S-methyl, iprodione and acibenzolar + iprodione inoculated with 0, 10^3, 10^4 and 10^5 *B. cinerea* conidia mL^{-1} and incubated at 5, 12 and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatment [a]		1	2	3	4	5	6	7	8	9	10	11	12	13
C 0 [b] 5 [c]	24	.0000												
A 0 5	24	.0000												
I 0 5	24	.0000												
I 0 12	24	.0000												
I 0 20	12	.0000												
AI 0 5	24	.0000												
AI 0 12	24	.0000												
AI 0 20	12	.0000												
AI 10^3 5	24	4.167E-02												
A 0 12	24	.1250												
C 0 12	24	.2083	.2083											
A 10^3 5	24	.3333	.3333	.3333										
A 0 20	12	.3333	.3333	.3333										
I 10^3 5	24	.3333	.3333	.3333										
I 10^3 12	24	.3333	.3333	.3333										
I 10^3 20	12	.5000	.5000	.5000	.5000									

A 10^3 12	24	.5417	.5417	.5417	.5417				
C 100 20	12	.5833	.5833	.5833	.5833				
AI 10^3 20	12	.5833	.5833	.5833	.5833				
C 10^3 5	24		.7500	.7500	.7500				
AI 10^3 12	24		.7500	.7500	.7500				
C 10^3 12	24			.8333	.8333	.8333			
A 10^3 20	12			.9167	.9167	.9167	.9167		
C 10^3 20	12				1.0833	1.0833	1.0833	1.0833	
AI 10^4 5	24					1.3750	1.3750	1.3750	1.3750
A 10^4 12	24						1.4167	1.4167	1.4167
A 10^4 20	12							1.5833	1.5833
A 10^4 5	24							1.6250	1.6250
I 10^4 5	24						1.6667	1.6667	
AI 10^4 20	12						1.6667	1.6667	
C 10^4 5	24							1.7500	
I 10^4 20	12							1.9167	1.9167
C 10^4 20	12							2.4167	2.4167
C 10^4 12	24							2.4583	2.4583
AI 10^4 12	24							2.4583	2.4583
AI 10^5 5	24								2.5417

I 10^4 12	24									2.6250			
A 10^5 5	24										3.1667		
A 10^5 12	24										3.2917	3.2917	
I 10^5 5	24										3.5833	3.5833	3.5833
AI 10^5 20	12										3.5833	3.5833	3.5833
AI 10^5 12	24										3.6250	3.6250	3.6250
I 10^5 12	24											3.8333	3.8333
C 10^5 5	24												3.8750
I 10^5 20	12												3.9167
C 10^5 12	24												4.0000
C 10^5 20	12												4.0000
A 10^5 20	12												4.0000
Sig.		.076	.087	.066	.059	.057	.080	.053	.078	.057	.488	.120	.064	.187

[a] C = control, A = acibenzolar, I = Iprodione, and AI = Acibenzolar + Iprodione.

[b] 10^3, 10^4 and 10^5 *B. cinerea* conidia mL^{-1}, respectively.

[c] 5 = 5, 12 = 12, and 20 = 20°C

A4.1.4 Effect of acibenzolar-S-methyl on vase life, fresh weight and wilt score (experiment A4)

Table A4.1.4.1: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	24	.000		
Total	.000	27			

Table A4.1.4.2: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 1).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.464E-02	3	1.488E-02	1.000	.410
Within Groups	.357	24	1.488E-02		
Total	.402	27			

Table A4.1.4.3: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.208	3	6.917E-02	1.395	0.269
Within Groups	1.190	24	4.958E-02		
Total	1.398	27			

Table A4.1.4.4: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 3).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.312	3	.104	1.087	.374
Within Groups	2.300	24	9.585E-02		
Total	2.613	27			

Table A4.1.4.5: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.435	3	1.478	11.898	.000
Within Groups	2.982	24	.124		
Total	7.417	27			

Table A4.1.4.6: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 5).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.268	3	8.929E-02	.467	.708
Within Groups	4.589	24	.191		
Total	4.857	27			

Table A4.1.4.7: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.125	3	.708	2.867	.058
Within Groups	5.929	24	.247		
Total	8.054	27			

Table A4.1.4.8: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 7).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.732	3	.244	1.471	.248
Within Groups	3.982	24	.166		
Total	4.714	27			

Table A4.1.4.9: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.759	3	.586	2.671	.070
Within Groups	5.268	24	.219		
Total	7.027	27			

Table A4.1.4.10: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 9).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.685	3	1.562	16.146	.000
Within Groups	2.321	24	9.673E-02		
Total	7.007	27			

Table A4.1.4.11: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 10).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.723	3	.241	2.817	.061
Within Groups	2.054	24	8.557E-02		
Total	2.777	27			

Table A4.1.4.12: One-way ANOVA table for wilt score of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 11).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.027E-02	3	2.009E-02	1.000	.410
Within Groups	.482	24	2.009E-02		
Total	.542	27			

Table A4.1.4.13: One-way ANOVA table for flower fresh weight of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	24	.000		
Total	.000	27			

Table A4.1.4.14: One-way ANOVA table for flower relative fresh weight of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.215	3	2.405	1.165	.344
Within Groups	49.548	24	20.64		
Total	56.763	27			

Table A4.1.4.15: One-way ANOVA table for flower relative fresh weight of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	40.572	3	13.524	2.305	.102
Within Groups	140.804	24	5.867		
Total	181.376	27			

Table A4.1.4.16: One-way ANOVA table for flower relative fresh weight of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.419	3	25.473	3.897	.021
Within Groups	156.870	24	6.536		
Total	233.289	27			

Table A4.1.4.17: One-way ANOVA table for flower relative fresh weight of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	113.337	3	37.779	1.517	.236
Within Groups	597.635	24	24.901		
Total	710.972	27			

Table A4.1.4.18: One-way ANOVA table for flower relative fresh weight of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 10).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	337.520	3	112.507	2.603	.075
Within Groups	1037.168	24	43.215		
Total	1374.688	27			

Table A4.1.4.19: One-way ANOVA table for flower relative fresh weight of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates (day 11).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	77.070	3	25.690	1.625	.210
Within Groups	379.502	24	15.813		
Total	456.572	27			

Table A4.1.4.20: One-way ANOVA table for vase lives of acibenzolar treated freesia var. 'Cote d'Azur' flowers at four rates.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.964	3	.321	1.350	.282
Within Groups	5.714	24	.238		
Total	6.679	27			

A4.1.5 Effect of acibenzolar-S-methyl on PAL activity (experiment A5)

Table A4.1.5.1: One-way ANOVA table for PAL activity of freesia var. 'Texel' treated with acibenzolar-S-methyl at 0.15 g AIL^{-1} or left untreated and incubated at 20°C (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	78439.353	1	78439.353	.794	.380
Within Groups	2764984.838	28	98749.459		
Total	2843424.192	29			

Table A4.1.5.2: One-way ANOVA table for PAL activity of freesia var. 'Texel' treated with acibenzolar-S-methyl at 0.15 g AIL^{-1} or left untreated and incubated at 20°C (day 1).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	753726.553	1	753726.553	3.195	.088
Within Groups	5189547.509	22	235888.523		
Total	5943274.063	23			

Table A4.1.5.3: One-way ANOVA table for PAL activity of freesia var. 'Texel' treated with acibenzolar-S-methyl at 0.15 g AIL^{-1} or left untreated and incubated at 20°C (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	117065.198	1	117065.198	2.661	.116
Within Groups	1011974.693	23	43998.900		
Total	1129039.891	24			

Table A4.1.5.4: One-way ANOVA table for PAL activity of freesia var. 'Texel' treated with acibenzolar-S-methyl at 0.15 g AIL^{-1} or left untreated and incubated at 20°C (day 3).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	581496.050	1	581496.050	2.494	.126
Within Groups	6529386.424	28	233192.372		
Total	7110882.474	29			

A4.1.6 Effect of acibenzolar-S-methyl on *B. cinerea* mycelium growth, conidial germination and germ tube elongation (experiment A6)

Table A4.1.6.1: One-way ANOVA table for conidial germination in 4 acibenzolar rates. Spore solutions were incubated for 12 h at 20°C in the dark.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	276.188	3	92.063	2.325	.127
Within Groups	475.250	12	39.604		
Total	751.438	15			

Table A4.1.6.2: One-way ANOVA table for *B. cinerea* germ tube elongation in 4 acibenzolar rates. Spore solutions were incubated for 12 h at 20°C in the dark.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7956.480	3	2652.160	13.960	.000
Within Groups	14438.400	76	189.979		
Total	22394.880	79			

Table A4.1.6.3: One-way ANOVA table for colony diameter of *B. cinerea* in media supplemented with 4 acibenzolar rates. Spore solutions were incubated for 12 h at 20°C in the dark.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	268.176	3	89.392	40.965	.000
Within Groups	209.486	96	2.182		
Total	477.662	99			

APPENDIX 4.2: SUPPRESSION BY POSTHARVEST TREATMENT WITH METHYL JASMONATE

A4.2.1 Effects of gaseous MeJA on artificially inoculated flowers (experiment M1)

Table A4.2.1.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12 and 20°C.

Treatments	N		Mean Rank
Control	36		100.79
MeJA1	36		71.54
MeJA2	36		63.63
MeJA3	36		54.04
Total	144		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	3	25.849	.000

Table A4.2.1.2: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12 and 20°C.

Temperature	N		Mean Rank
5	48		45.90
12	48		100.19
20	48		71.42
Total	144		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	41.657	.000

Table A4.2.1.3: ANOVA table for disease severity of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	42.750	11	3.886	13.861	.000
Intercept	213.160	1	213.160	760.257	.000
CHEMICAL TREATMENT (C)	15.048	3	5.016	17.890	.000
TEMPERATURE (T)	23.845	2	11.923	42.524	.000
C * T	3.856	6	.643	2.292	.039
Error	37.010	132	.280		
Total	292.920	144			
Corrected Total	79.760	143			

Table A4.2.1.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4	5
MeJA 3 5 [b]	12	.6417				
MeJA 2 5	12	.6667				
MeJA 1 5	12	.7167				
MeJA 3 20	12	.8250	.8250			
Control 5	12	.9000	.9000			
MeJA 2 20	12	.9833	.9833			
MeJA 1 20	12	1.0750	1.0750	1.0750		
MeJA 3 12	12		1.2000	1.2000		
MeJA 2 12	12			1.5000	1.5000	
MeJA 1 12	12				1.7500	
Control 20	12				1.8833	
Control 12	12					2.4583
Sig.		.085	.125	.062	.094	1.000

[a] MeJA 1, 2, 3 = 0.025, 0.05 and 0.1 μL L^{-1}, respectively. Control = 0 μL L^{-1}.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.2.1.5: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	52877.021	11	4807.002	18.877	.000
Intercept	257810.063	1	257810.063	1012.406	.000
CHEMICAL TREATMENT (C)	12755.910	3	4251.970	16.697	.000
TEMPERATURE (T)	37422.375	2	18711.188	73.478	.000
C * T	2698.736	6	449.789	1.766	.111
Error	33613.917	132	254.651		
Total	344301.000	144			
Corrected Total	86490.937	143			

Table A4.2.1.6: Lesion number means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4	5	6
MeJA 2 5 [b]	12	15.2500					
MeJA 3 5	12	16.9167					
MeJA 1 5	12	22.2500					
Control 5	12	29.0833	29.0833				
MeJA 1 20	12		36.8333	36.8333			
MeJA 3 20	12		36.9167	36.9167			
MeJA 2 20	12		41.4167	41.4167			
MeJA 3 12	12			47.6667	47.6667		
MeJA 2 12	12				57.4167	57.4167	
MeJA 1 12	12				58.3333	58.3333	
Control 20	12					70.0833	70.0833
Control 12	12						75.5833
Sig.		.051	.084	.131	.122	.065	.399

[a] MeJA 1, 2, 3 = 0.025, 0.05 and 0.1 μL L^{-1}, respectively. Control = 0 μL L^{-1}.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.2.1.7: ANOVA table for lesion diameter of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.877	11	.443	14.966	.000
Intercept	62.160	1	62.160	2098.329	.000
CHEMICAL TREATMENT (C)	2.229	3	.743	25.085	.000
TEMPERATURE (T)	.687	2	.343	11.595	.000
C * T	1.341	6	.223	7.543	.000
Error	3.081	104	2.962E-02		
Total	72.221	116			
Corrected Total	7.958	115			

Table A4.2.1.8: Lesion diameter means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4	5
MeJA 3 20 [b]	10	.5000				
MeJA 2 20	10	.5820	.5820			
MeJA 3 5	10	.5850	.5850			
Control 5	6	.5983	.5983			
MeJA 3 12	10	.6480	.6480	.6480		
MeJA 2 12	10	.6680	.6680	.6680		
MeJA 1 5	10	.6750	.6750	.6750		
MeJA 2 5	10		.7150	.7150		
MeJA 1 12	10			.8010		
MeJA 1 20	10			.8100		
Control 20	10				1.0640	
Control 12	10					1.2270
Sig.		.057	.153	.075	1.000	1.000

[a] MeJA 1, 2, 3 = 0.025, 0.05 and 0.1 μL L^{-1}, respectively. Control = 0 μL L^{-1}.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.2.1.9: Parameters estimated for the linear model ($y = y_0 + ax + bx^2$) used to describe the effects of gaseous MeJA concentration and incubation temperature on disease severity, lesion number and lesion diameter on freesia petals.

Temperature (°C)	Estimated parameters			Coefficient (R^2)
	y_0	a	b	
a. Disease severity				
5	0.88	−6.02	32.73	0.90
12	2.48	−28.3	156.36	0.99
20	1.84	−28.05	178.18	0.94
b. Lesion number				
5	29.44	−416.73	2909	0.98
12	74.40	−564.36	3054.55	0.93
20	66.56	−983.27	6690	0.83
c. Lesion diameter				
5	0.70	−0.33	−2.91	0.49
12	1.088	−13.33	89.82	0.99
20	0.99	−11.25	64.36	0.99

Table A4.2.1.10: One-way ANOVA table for lesion diameter of detached petals. Freesia var. 'Cote d'Azur' flowers were gassed with four MeJA concentrations.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.650	3	6.550	5.712	.003
Within Groups	41.284	36	1.147		
Total	60.934	39			

A4.2.2 Effects of gaseous MeJA on un-inoculated (naturally infected) flowers (experiment M2)

Table A4.2.2.1: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at three rates in un-inoculated (naturally infected) flowers incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	110.139	11	10.013	2.895	.002
Intercept	367.361	1	367.361	106.225	.000
CHEMICAL TREATMENT (C)	17.917	3	5.972	1.727	.165
TEMPERATURE (T)	5.681	2	2.840	.821	.442
C * T	86.542	6	14.424	4.171	.001
Error	456.500	132	3.458		
Total	934.000	144			
Corrected Total	566.639	143			

A4.2.3 Effects of gaseous MeJA on freesia vase life (experiment M3)

Table A4.2.3.1: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	24	.000		
Total	.000	27			

Table A4.2.3.2: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 1).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.750E-02	3	1.250E-02	2.667	.083
Within Groups	7.500E-02	16	4.688E-03		
Total	.112	19			

Table A4.2.3.3: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.309	3	.103	6.600	.004
Within Groups	.250	16	1.563E-02		
Total	.559	19			

Table A4.2.3.4: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 3).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.934	3	.311	5.111	.011
Within Groups	.975	16	6.094E-02		
Total	1.909	19			

Table A4.2.3.5: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.913	3	.638	3.290	.048
Within Groups	3.100	16	.194		
Total	5.013	19			

Table A4.2.3.6: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 5).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.784	3	1.261	3.541	.039
Within Groups	5.700	16	.356		
Total	9.484	19			

Table A4.2.3.7: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.559	3	.853	2.405	.105
Within Groups	5.675	16	.355		
Total	8.234	19			

Table A4.2.3.8: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 7).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.300	3	.433	1.284	.314
Within Groups	5.400	16	.338		
Total	6.700	19			

Table A4.2.3.9: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.313	3	.438	1.582	.233
Within Groups	4.425	16	.277		
Total	5.738	19			

Table A4.2.3.10: One-way ANOVA table for wilt score of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 9).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	16	.000		
Total	.000	19			

Table A4.2.3.11: One-way ANOVA table for flower fresh weight of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	24	.000		
Total	.000	27			

Table A4.2.3.12: One-way ANOVA table for flower relative fresh weight of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.811	3	2.270	.311	.817
Within Groups	116.886	16	7.305		
Total	123.698	19			

Table A4.2.3.13: One-way ANOVA table for flower relative fresh weight of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	136.725	3	45.575	2.692	.081
Within Groups	270.892	16	16.931		
Total	407.618	19			

Table A4.2.3.14: One-way ANOVA table for flower relative fresh weight of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	506.932	3	168.977	6.202	.005
Within Groups	435.918	16	27.245		
Total	942.850	19			

Table A4.2.3.15: One-way ANOVA table for flower relative fresh weight of v treated freesia var. 'Cote d'Azur' flowers at four rates (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	538.786	3	179.595	3.424	.043
Within Groups	839.272	16	52.455		
Total	1378.058	19			

Table A4.2.3.16: One-way ANOVA table for flower relative fresh weight of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates (day 10).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	422.125	3	140.708	2.883	.068
Within Groups	780.804	16	48.800		
Total	1202.929	19			

Table A4.2.3.17: One-way ANOVA table for vase lives of MeJA gas treated freesia var. 'Cote d'Azur' flowers at four rates.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.350	3	1.117	2.481	.098
Within Groups	7.200	16	.450		
Total	10.550	19			

A4.2.4 Effect of pulse MeJA on artificially inoculated flowers (experiment M4)

Table A4.2.4.1: Non-parametric test (Kruskal-Wallis) for disease severity rating scale of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia $L^{-1}$and incubated at 5, 12 and 20°C.

Treatments	N		Mean Rank
Control	40		92.64
MeJA1	40		66.61
MeJA2	40		79.66
MeJA3	40		83.09
Total	160		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	3	6.558	.087

Table A4.2.4.2: Non-parametric test (Kruskal-Wallis) for disease severity rating scale of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia $L^{-1}$and incubated at 5, 12 and 20°C.

Temperature (°C)	N		Mean Rank
5	60		39.77
12	60		104.49
20	40		105.61
Total	160		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	75.142	.000

Table A4.2.4.3: ANOVA table for disease severity of freesia var. 'var. 'Cote d'Azur' flowers pulsed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	118.724	11	10.793	14.920	.000
Intercept	483.665	1	483.665	668.591	.000
CHEMICAL TREATMENT (C)	12.262	3	4.087	5.650	.001
TEMPERATURE (T)	102.145	2	51.072	70.599	.000
C * T	5.971	6	.995	1.376	.228
Error	107.065	148	.723		
Total	689.890	160			
Corrected Total	225.788	159			

Table A4.2.4.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers pulsed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4	5
MeJA 3 5 [b]	15	.6067				
MeJA 1 5	15	.6267				
Control 5	15	.7200				
MeJA 2 5	15	.7333				
MeJA 1 20	10		1.6600			
MeJA 1 12	15		1.8400	1.8400		
MeJA 20	10		2.1000	2.1000	2.1000	
MeJA 12	15		2.2400	2.2400	2.2400	2.2400
MeJA 12	15			2.4467	2.4467	2.4467
MeJA 20	10			2.5300	2.5300	2.5300
Control 12	15				2.7933	2.7933
Control 20	10					2.9500
Sig.		.736	.116	.066	.065	.058

[a] MeJA 1, 2, 3 = 200, 400 and 600 µM, respectively. Control = 0 µM.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.2.4.5: ANOVA table for lesion number of freesia var. 'var. 'Cote d'Azur' flowers pulsed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	98654.775	11	8968.616	18.321	.000
Intercept	471535.243	1	471535.243	963.273	.000
CHEMICAL TREATMENT (C)	8708.624	3	2902.875	5.930	.001
TEMPERATURE (T)	86754.108	2	43377.054	88.613	.000
C * T	3995.942	6	665.990	1.361	.234
Error	72448.000	148	489.514		
Total	619060.000	160			
Corrected Total	171102.775	159			

Table A4.2.4.6: Lesion number means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers pulsed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatment [a]	N	1	2	3	4
MeJA 2 5 [b]	15	20.9333			
MeJA 1 5	15	23.2000			
MeJA 3 5	15	23.7333			
Control 5	15	24.0667			
MeJA 1 12	15		56.0000		
MeJA 2 12	15		62.6667	62.6667	
MeJA 2 20	10		63.1000	63.1000	
MeJA 1 20	10		63.5000	63.5000	
MeJA 3 12	15		71.9333	71.9333	71.9333
MeJA 3 20	10			80.5000	80.5000
Control 12	15				83.8667
Control 20	10				89.9000
Sig.		.749	.105	.068	.059

[a] MeJA 1, 2, 3 = 200, 400 and 600 µM, respectively. Control = 0 µM.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.2.4.7: ANOVA table for lesion diameter of freesia var. 'var. 'Cote d'Azur' flowers treated pulsed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.123	11	.193	5.254	.000
Intercept	74.170	1	74.170	2019.490	.000
CHEMICAL TREATMENT (C)	.519	3	.173	4.714	.004
TEMPERATURE (T)	.934	2	.467	12.709	.000
C * T	.478	6	7.965E-02	2.169	.050
Error	4.701	128	3.673E-02		
Total	86.537	140			
Corrected Total	6.824	139			

Table A4.2.4.8: Lesion diameter means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated pulsed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4
MeJA 3 5 [b]	10	.5910			
MeJA 1 5	10	.6310			
Control 5	10	.6620	.6620		
MeJA 2 5	10	.6660	.6660		
MeJA 1 20	10	.6850	.6850	.6850	
MeJA 2 12	15	.7180	.7180	.7180	
MeJA 3 12	15	.7180	.7180	.7180	
MeJA 2 20	10	.7390	.7390	.7390	
MeJA 3 20	10	.7440	.7440	.7440	
Control 20	10		.8380	.8380	
MeJA 1 12	15			.8667	
Control 12	15				1.0360
Sig.		.113	.063	.051	1.000

[a] MeJA 1, 2, 3 = 200, 400 and 600 µM, respectively. Control = 0 µM.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.2.4.9: Parameters estimated for the linear model ($y = y_0 + ax + bx^2$) used to describe the effects of MeJA pulse concentration and incubation temperature on disease severity, lesion number and lesion diameter on freesia petals.

Temperature (°C)	Estimated parameters			Coefficient (R^2)
	y_0	a	b	
a. Disease severity				
5	0.68	−0.0001	0	0.2
12	2.72	−0.005	0	0.75
20	2.90	−0.007	0	0.84
b. Lesion number				
5	23.3	−0.001	0	0.03
12	82.35	−0.15	0	0.88
20	89.70	−0.18	0	0.99
c. Lesion diameter				
5	0.65	0.0001	0	0.53
12	1.05	−0.001	0	0.99
20	0.83	-0.0007	0	0.74

Table A4.2.4.10: One-way ANOVA table for lesion diameter of detached petals. Freesia var. 'Cote d'Azur' flowers were pulsed with four MeJA concentrations.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.347	3	2.449	3.272	.032
Within Groups	26.942	36	.748		
Total	34.289	39			

4.2.5 Effect of pulse MeJA on un-inoculated (naturally infected) flowers (experiment M5)

Table A4.2.51: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with pulse MeJA at three rates, left un-inoculated (natural infection) and incubated at 5, 12 and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	262.360	11	23.851	4.132	.000
Intercept	1845.268	1	1845.268	319.701	.000
CHEMICAL TREATMENT (C)	104.985	3	34.995	6.063	.001
TEMPERATURE (T)	43.752	2	21.876	3.790	.025
C * T	100.090	6	16.682	2.890	.011
Error	854.233	148	5.772		
Total	2973.000	160			
Corrected Total	1116.594	159			

4.2.6 Effect of pulse MeJA on freesia vase life (experiment M6)

Table A4.2.6.1: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	24	.000		
Total	.000	27			

Table A4.2.6.2: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 1).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.969E-02	3	9.896E-03	.613	.611
Within Groups	.581	36	1.615E-02		
Total	.611	39			

Table A4.2.6.3: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.969E-02	3	9.896E-03	1.326	.281
Within Groups	.269	36	7.465E-03		
Total	.298	39			

Table A4.2.6.4: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 3).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.750E-02	3	2.917E-02	.730	.541
Within Groups	1.438	36	3.993E-02		
Total	1.525	39			

Table A4.2.6.5: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.213	3	7.083E-02	.716	.549
Within Groups	3.563	36	9.896E-02		
Total	3.775	39			

Table A4.2.6.6: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 5).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.713	3	.571	4.384	.010
Within Groups	4.688	36	.130		
Total	6.400	39			

Table A4.2.6.7: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.242	3	.747	3.414	.028
Within Groups	7.881	36	.219		
Total	10.123	39			

Table A4.2.6.8: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 7).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.306	3	.435	2.454	.079
Within Groups	6.388	36	.177		
Total	7.694	39			

Table A4.2.6.9: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.017	3	.339	7.051	.001
Within Groups	1.731	36	4.809E-02		
Total	2.748	39			

Table A4.2.6.10: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 9).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.381	3	.127	5.304	.004
Within Groups	.863	36	2.396E-02		
Total	1.244	39			

Table A4.2.6.11: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 10).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	36	.000		
Total	.000	39			

Table A4.2.6.12: One-way ANOVA table for flower fresh weight of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	24	.000		
Total	.000	27			

Table A4.2.6.13: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.573	3	.191	.112	.952
Within Groups	61.268	36	1.702		
Total	61.840	39			

Table A4.2.6.14: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	59.504	3	19.835	2.828	.052
Within Groups	252.502	36	7.014		
Total	312.005	39			

Table A4.2.6.15: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	392.511	3	130.837	3.761	.019
Within Groups	1252.483	36	34.791		
Total	1644.994	39			

Table A4.2.6.16: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2292.272	3	764.091	9.431	.000
Within Groups	2916.827	36	81.023		
Total	5209.098	39			

Table A4.2.6.17: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates (day 10).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1128.586	3	376.195	3.556	.024
Within Groups	3808.212	36	105.784		
Total	4936.798	39			

Table A4.2.6.18: One-way ANOVA table for vase lives of freesia var. 'Cote d'Azur' flowers pulsed with MeJA at four rates.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.600	3	2.867	7.478	.001
Within Groups	13.800	36	.383		
Total	22.400	39			

4.2.7 Effect of MeJA applied as spray on artificially inoculated freesia flowers (experiment M7)

Table A4.2.7.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12 and 20°C.

Treatments	N		Mean Rank
Control	36		86.74
MeJA1	36		75.25
MeJA2	35		66.73
MeJA3	36		59.14
Total	143		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	3	8.955	.030

Table A4.2.7.2: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia L^{-1} and incubated at 5, 12 and 20°C.

Temperature (°C)	N	Mean Rank
5	48	27.86
12	48	104.97
20	47	83.40
Total	143	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	89.864	.000

Table A4.2.7.3: ANOVA table for disease severity of freesia var. 'var. 'Cote d'Azur' flowers sprayed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	66.153	11	6.014	23.212	.000
Intercept	248.286	1	248.286	958.322	.000
CHEMICAL TREATMENT (C)	5.205	3	1.735	6.697	.000
TEMPERATURE (T)	58.575	2	29.287	113.042	.000
C * T	2.328	6	.388	1.498	.184
Error	33.940	131	.259		
Total	348.570	143			
Corrected Total	100.093	142			

Table A4.2.7.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers sprayed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5	6
MeJA 1 5 [b]	12	.4250					
MeJA 3 5	12	.4500					
MeJA 2 5	12	.4750					
Control 5	12	.5250					
MeJA 3 20	12		1.0750				
MeJA 2 20	11		1.3000	1.3000			
MeJA 1 20	12			1.6000	1.6000		
MeJA 3 12	12			1.7250	1.7250	1.7250	
MeJA 2 12	12				1.8917	1.8917	1.8917
Control 20	12				1.9417	1.9417	1.9417
MeJA 1 12	12					2.0833	2.0833

Control 12	12						2.3250
Sig.		.669	.281	.053	.137	.118	.057

[a] MeJA 1, 2, 3 = 200, 400 and 600 μM, respectively. Control = 0 μM.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.2.7.5: ANOVA table for lesion number of freesia var. 'var. 'Cote d'Azur' flowers treated sprayed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	66541.040	11	6049.185	32.793	.000
Intercept	283188.659	1	283188.659	1535.205	.000
CHEMICAL TREATMENT (C)	6798.442	3	2266.147	12.285	.000
TEMPERATURE (T)	57275.594	2	28637.797	155.249	.000
C * T	2373.532	6	395.589	2.145	.052
Error	24164.667	131	184.463		
Total	373659.000	143			
Corrected Total	90705.706	142			

Table A4.2.7.6: Lesion number means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers sprayed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4	5	6
MeJA 2 5 [b]	12	14.7500					
MeJA 1 5	12	15.6667					
MeJA 3 5	12	16.6667					
Control 5	12	20.0000					
MeJA 3 20	12		42.9167				
MeJA 2 20	11		49.0000	49.0000			
MeJA 3 12	12		52.5000	52.5000	52.5000		
MeJA 2 12	12			57.0833	57.0833	57.0833	
MeJA 1 20	12			57.3333	57.3333	57.3333	
MeJA 1 12	12				61.8333	61.8333	
Control 20	12					66.2500	
Control 12	12						80.1667
Sig.		.398	.104	.175	.128	.135	1.000

[a] MeJA 1, 2, 3 = 200, 400 and 600 μM, respectively. Control = 0 μM.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.2.7.7: ANOVA table for lesion diameter of freesia var. 'var. 'Cote d'Azur' flowers treated sprayed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.998	11	.909	2.566	.004
Intercept	158.578	1	158.578	447.669	.000
CHEMICAL TREATMENT (C)	1.489	3	.496	1.401	.243
TEMPERATURE (T)	5.043	2	2.521	7.118	.001
C * T	2.964	6	.494	1.395	.217
Error	87.849	248	.354		
Total	273.525	260			
Corrected Total	97.847	259			

Table A4.2.7.8: Lesion diameter means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers sprayed with MeJA at three rates, inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2
MeJA 2 5 [b]	15	.6420	
MeJA 1 5	15	.6567	
MeJA 2 20	25	.6820	
MeJA 3 5	15	.6867	
MeJA 1 20	25	.6992	
MeJA 3 20	25	.7248	
Control 5	15	.7560	
MeJA 2 12	25	.7920	
Control 20	25	.8104	
MeJA 3 12	25	.8480	
Control 12	25	1.0048	1.0048
MeJA 1 12	25		1.3428
Sig.		.109	.069

[a] MeJA 1, 2, 3 = 200, 400 and 600 µM, respectively. Control = 0 µM.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.2.7.9: Parameters estimated for the linear model ($y = y_0 + ax + bx^2$) used to describe the effects of MeJA spray concentration and incubation temperature on disease severity, lesion number and lesion diameter on freesia petals.

Temperature (°C)	Estimated parameters			Coefficient (R^2)
	y_0	a	b	
a. Disease severity				
5	0.46	0.0001	0	0.07
12	1.88	−0.014	0	0.99
20	2.30	−0.001	0	1
b. Lesion number				
5	20.00	−0.0275	0	1
12	79.4	−0.10	0.0001	0.98
20	66.1	−0.05	0	1
c. Lesion diameter				
5	0.73	−0.001	0	0.52
12	1.11	−0.0014	0	0.99
20	0.77	−0.0007	0	0.96

Table A4.2.7.10: One-way ANOVA table for lesion diameter of detached petals. Freesia var. 'Cote d'Azur' flowers were pulsed with four MeJA concentrations.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.624	3	1.541	2.944	.046
Within Groups	18.847	36	.524		
Total	23.471	39			

4.2.8 Effect of MeJA applied as spray on un-inoculated flowers (experiment M8)

Table A4.2.8.1: ANOVA table for lesion number of freesia var. 'var. 'Cote d'Azur' flowers treated sprayed with MeJA at three rates, left un-inoculated (naturally infected) and incubated at 5, 12 and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	54.806	11	4.982	3.370	.000
Intercept	148.028	1	148.028	100.118	.000
CHEMICAL TREATMENT (C)	41.472	3	13.824	9.350	.000
TEMPERATURE (T)	6.764	2	3.382	2.287	.106
C * T	6.569	6	1.095	.741	.618
Error	195.167	132	1.479		
Total	398.000	144			
Corrected Total	249.972	143			

4.2.9 Effect of MeJA applied as spray on freesia vase life (experiment M9)

Table A4.2.9.1: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	24	.000		
Total	.000	27			

Table A4.2.9.2: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 1).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.437E-02	3	1.146E-02	1.467	.261
Within Groups	.125	16	7.813E-03		
Total	.159	19			

Table A4.2.9.3: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.213	3	7.083E-02	5.037	.012
Within Groups	.225	16	1.406E-02		
Total	.438	19			

Table A4.2.9.4: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 3).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.862	3	.287	11.500	.000
Within Groups	.400	16	2.500E-02		
Total	1.262	19			

Table A4.2.9.5: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.259	3	8.646E-02	.769	.528
Within Groups	1.800	16	.113		
Total	2.059	19			

Table A4.2.9.6: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 5).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.937	3	.646	3.255	.049
Within Groups	3.175	16	.198		
Total	5.112	19			

Table A4.2.9.7: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.050	3	.350	1.014	.413
Within Groups	5.525	16	.345		
Total	6.575	19			

Table A4.2.9.8: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 7).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.112	3	.371	.913	.457
Within Groups	6.500	16	.406		
Total	7.613	19			

Table A4.2.9.9: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.134	3	4.479E-02	1.509	.250
Within Groups	.475	16	2.969E-02		
Total	.609	19			

Table A4.2.9.10: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 9).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	16	.000		
Total	.000	19			

Table A4.2.9.11: One-way ANOVA table for flower fresh weight of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	16	.000		
Total	.000	19			

Table A4.2.9.12: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	49.715	3	16.572	3.476	.041
Within Groups	76.273	16	4.767		
Total	125.989	19			

Table A4.2.9.13: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	191.891	3	63.964	4.076	.025
Within Groups	251.092	16	15.693		
Total	442.982	19			

Table A4.2.9.14: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	256.016	3	85.339	2.747	.077
Within Groups	497.068	16	31.067		
Total	753.083	19			

Table A4.2.9.15: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	297.106	3	99.035	2.521	.095
Within Groups	628.515	16	39.282		
Total	925.621	19			

Table A4.2.9.16: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates (day 10).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	252.931	3	84.310	1.850	.179
Within Groups	729.309	16	45.582		
Total	982.240	19			

Table A4.2.9.17: One-way ANOVA table for vase lives of freesia var. 'Cote d'Azur' flowers sprayed with MeJA at four rates.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.000	3	.333	1.905	.170
Within Groups	2.800	16	.175		
Total	3.800	19			

4.2.10 Effect of MeJA on mycelial growth, conidial germination and germ tube elongation (experiment M10)

Table A4.2.10.1: One-way ANOVA table for conidial germination in four MeJA rates. Conidial solutions were incubated for 12 h at 20°C in the dark.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1038.688	3	346.229	29.003	.000
Within Groups	143.250	12	11.938		
Total	1181.938	15			

Table A4.2.10.2: One-way ANOVA table for *B. cinerea* germ tube elongation in four MeJA rates. Conidial solutions were incubated for 12 h at 20°C in the dark.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1078.656	3	359.552	1.223	.307
Within Groups	22334.976	76	293.881		
Total	23413.632	79			

Table A4.2.10.3: One-way ANOVA table for colony diameter of *B. cinerea* in media supplemented with four MeJA rates. Inoculated media were incubated at 20°C in the dark for five days.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	39.757	3	13.252	2.170	.097
Within Groups	586.173	96	6.106		
Total	625.930	99			

APPENDIX 4.3: FURTHER ASSESSMENT OF POSTHARVEST TREATMENT WITH GASEOUS METHYL JASMONATE ON INDUCED DEFENCE RESPONSES

4.3.1 Effect of gaseous MeJA on artificially inoculated flowers (experiment Mg1)

Table A4.3.1.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers were inoculated at 0 h with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 48 h in the dark.

Treatments	N		Mean Rank
Control	100		112.53
MeJA	100		88.47
Total	200		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	1	9.336	.002

Table A4.3.1.2: Non-parametric test (Kruskal-Wallis) for disease severity of gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers were inoculated at 0 h with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 48 h in the dark.

Time	N		Mean Rank
0 h	40		42.00
6 h	40		42.00
12 h	40		109.81
24 h	40		148.75
48 h	40		159.94
Total	200		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	4	165.045	.000

Table A4.3.1.3: ANOVA table for disease severity of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers were inoculated at 0 h with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 48 h in the dark.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	329.143	9	36.571	211.232	.000
Intercept	376.751	1	376.751	2176.065	.000
CHEMICAL TREATMENT (C)	19.908	1	19.908	114.986	.000
TIME (T)	293.156	4	73.289	423.307	.000
C * T	16.079	4	4.020	23.218	.000
Error	32.896	190	.173		
Total	738.790	200	.		
Corrected Total	362.039	199			

Table A4.3.1.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers were inoculated at 0 h with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 48 h in the dark. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4
Control 0 h	20	.0000			
Control 6 h	20	.0000			
MeJA 0 h	20	.0000			
MeJA 6 h	20	.0000			
MeJA 12 h	20		.7200		
MeJA 24 h	20			2.1500	
Control 12 h	20			2.2000	
MeJA 48 h	20			2.4150	
Control 24 h	20				3.0400
Control 48 h	20				3.2000
Sig.		1.000	1.000	.056	.224

[a] MeJA = 0.1 μL L^{-1}. Control = 0 μL L^{-1}.

Table A4.3.1.5: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers were inoculated at 0 h with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 48 h in the dark.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	320501.180	9	35611.242	145.994	.000
Intercept	400333.520	1	400333.520	1641.232	.000
CHEMICAL TREATMENT (C)	300996.030	4	75249.008	308.495	.000
TIME (T)	9302.480	1	9302.480	38.137	.000
C * T	10202.670	4	2550.667	10.457	.000
Error	46345.300	190	243.923		
Total	767180.000	200			
Corrected Total	366846.480	199			

Table A4.3.1.6: Lesion number means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers were inoculated at 0 h with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 48 h in the dark. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Control 0 h	20	.0000				
Control 6 h	20	.0000				
MeJA 0 h	20	.0000				
MeJA 6h	20	.0000				
MeJA 12 h	20		31.4500			
Control 12 h	20			70.0000		
MeJA 48 h	20			77.4500	77.4500	
MeJA 24 h	20				80.7000	
Control 24 h	20					92.0000
Control 48 h	20					95.8000
Sig.		1.000	1.000	.131	.511	.442

[a] MeJA = 0.1 μL L^{-1}. Control = 0 μL L^{-1}.

Table A4.3.1.7: ANOVA table for lesion diameter of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers were inoculated at 0 h with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 48 h in the dark.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19.050	9	2.117	248.347	.000
Intercept	21.924	1	21.924	2572.285	.000
CHEMICAL TREATMENT (C)	1.607	1	1.607	188.596	.000
TIME (T)	16.902	4	4.225	495.762	.000
C * T	1.230	4	.307	36.077	.000
Error	1.108	130	8.523E-03		
Total	32.687	140			
Corrected Total	20.158	139			

Table A4.3.1.8: Lesion diameter means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers were inoculated at 0 h with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C for 48 h in the dark. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Control 0 h	20	.0000				
Control 6 h	20	.0000				
MeJA 0 h	20	.0000				
MeJA 6 h	20	.0000				
MeJA 12 h	10		.4610			
MeJA 24 h	10		.4860			
MeJA 48 h	10			.5800		
Control 24 h	10				.8390	
Control 12 h	10				.8440	
Control 48 h	10					.9780
Sig.		1.000	.498	1.000	.892	1.000

[a] MeJA = 0.1 μL L^{-1}. Control = 0 μL L^{-1}.

4.3.2 Effect of gaseous MeJA on un-inoculated (naturally infected) flowers (experiment Mg 2)

Table A4.3.2.1: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers were left un-inoculated (naturally infected).

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1867.345	9	207.483	14.358	.000
Intercept	1794.005	1	1794.005	124.146	.000
CHEMICAL TREATMENT (C)	361.805	1	361.805	25.037	.000
TIME (T)	1264.070	4	316.018	21.869	.000
C * T	241.470	4	60.368	4.177	.003
Error	2745.650	190	14.451		
Total	6407.000	200			
Corrected Total	4612.995	199			

Table A4.3.2.2: Lesion number means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. The flowers left un-inoculated (naturally infected). Numbers on the same column are not significantly different at P = 0.05

Treatments		1	2	3
Control 0 h	20	.0000		
Control 6 h	20	.0000		
MeJA 0 h	20	.0000		
MeJA 6 h	20	.0000		
MeJA 12 h	20	1.8000	1.8000	
MeJA 24 h	20		2.9500	
MeJA 48 h	20		3.5000	
Control 12 h	20			6.1500
Control 24 h	20			7.5000
Control 48 h	20			8.0500
Sig.		.187	.184	.136

4.3.3 PPO activity in gaseous MeJA treated flowers (experiment Mg 3)

Table A4.3.3.1: ANOVA table for PPO activity in freesia var. 'Cote d'Azur' flowers treated with MeJA at 0.1 μL L^{-1} air or left untreated for 24 h at 20°C and inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.471	19	2.479E-02	4.802	.000
Intercept	2.419	1	2.419	468.687	.000
CHEMICAL TREATM (C)	5.905E-02	1	5.905E-02	11.439	.002
TIME (T)	.215	4	5.363E-02	10.389	.000
INOCULUM (I)	2.931E-02	1	2.931E-02	5.677	.022
C * T	.110	4	2.761E-02	5.349	.002
C * I	2.008E-02	1	2.008E-02	3.890	.056
T * I	2.363E-02	4	5.907E-03	1.144	.350
C * T * I	1.391E-02	4	3.479E-03	.674	.614
Error	.206	40	5.162E-03		
Total	3.097	60			
Corrected Total	.677	59			

Table A4.3.3.2: PPO activity g.f.w.$^{-1}$ min^{-1} means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} or left un-inoculated and samples were taken at 0, 6, 12, 24, and 48h after treatment. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4
MeJA 12h +Bc [b]	3	.1049			
Control 24h -Bc	3	.1144			
MeJA 24h +Bc	3	.1290			
Control 6h +Bc	3	.1391			
Control 12h +Bc	3	.1421			
MeJA 48h +Bc	3	.1503	.1503		
MeJA 24h -Bc	3	.1510	.1510		
Control 12h -Bc	3	.1520	.1520		
Control 48h +Bc	3	.1625	.1625		
Control 6h -Bc	3	.1664	.1664		
Control 24h +Bc	3	.1739	.1739		
MeJA 6h +Bc	3	.1808	.1808		
Control 0h -Bc	3	.1965	.1965		
Control 0h +Bc	3	.2105	.2105		
MeJA 48h -Bc	3	.2187	.2187		
Control 48h -Bc	3	.2370	.2370		

MeJA 6h -Bc	3	.2412	.2412		
MeJA 12h -Bc	3		.2904	.2904	
MeJA 0h +Bc	3			.3939	.3939
MeJA 0h -Bc	3				.4616
Sig.		.061	.052	.085	.256

[a] MeJA = 0.1 μL L^{-1}. Control = 0 μL L^{-1}.

[b] ± Bc = ± *B. cinerea*

4.3.4 PAL activity in gaseous MeJA treated flowers (experiment Mg 4)

Table A4.3.4.1: ANOVA table for PAL activity in freesia var. 'Cote d'Azur' flowers treated with MeJA at 0.1 μL L^{-1} air or left untreated for 24 h at 20°C and inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11357625.123	19	597769.743	4.525	.000
Intercept	33940654.496	1	33940654.496	256.913	.000
CHEMICAL TREATM (C)	959514.081	1	959514.081	7.263	.008
TIME (T)	3115579.603	4	778894.901	5.896	.000
INOCULUM (I)	142430.169	1	142430.169	1.078	.301
C * T	3659602.142	4	914900.535	6.925	.000
C * I	326251.457	1	326251.457	2.470	.118
T * I	996437.578	4	249109.395	1.886	.116
C * T * I	2834706.031	4	708676.508	5.364	.000
Error	19552221.546	148	132109.605		
Total	69304898.882	168			
Corrected Total	30909846.669	167			

Table A4.3.4.2: PAL activity (nmoles *trans*-cinnamic acid g.f.w.$^{-1}$ h^{-1}) means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with gaseous MeJA at 0.1 μL L^{-1} air or left untreated for 24 h. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} or left un-inoculated and samples were taken at 0, 6, 12, 24, and 48h after treatment. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4	5	6	7	8
MeJA 24h +Bc [b]	6	.0000							
MeJA 48h -Bc	6	.0000							
MeJA 48h +Bc	6	.0000							
MeJA 12h +Bc	9	7.8889	7.8889						
Control 6h -Bc	9	33.8889	33.8889	33.8889					
Control 48h +Bc	9	37.1111	37.1111	37.1111	37.1111				
Control 6h +Bc	9		49.2222	49.2222	49.2222	49.2222			
Control 24h -Bc	9		52.2222	52.2222	52.2222	52.2222	52.2222		
MeJA 24h -Bc	9		52.8889	52.8889	52.8889	52.8889	52.8889		
Control 0h +Bc	9			58.6667	58.6667	58.6667	58.6667	58.6667	
MeJA 12h -Bc	9			65.5556	65.5556	65.5556	65.5556	65.5556	
MeJA 6h +Bc	9			71.6667	71.6667	71.6667	71.6667	71.6667	71.6667
MeJA 6h -Bc	9			72.6667	72.6667	72.6667	72.6667	72.6667	72.6667
MeJA 0h -Bc	9			77.3333	77.3333	77.3333	77.3333	77.3333	77.3333
MeJA 0h +Bc	9			82.4444	82.4444	82.4444	82.4444	82.4444	82.4444
Control 0h -Bc	9				84.3333	84.3333	84.3333	84.3333	84.3333
Control 12h -Bc	6					87.8333	87.8333	87.8333	87.8333
Control 24h +Bc	9						98.6667	98.6667	98.6667
Control 12h +Bc	9							102.3333	102.3333
Control 48h -Bc	9								115.4444
Sig.		.121	.057	.051	.059	.128	.064	.080	.076

[a] MeJA = 0.1 μL L^{-1}. Control = 0 μL L^{-1}.

[b] ± Bc = ± *B. cinerea*

APPENDIX 4.4: EFFECTS OF COMBINED TREATMENT OF ACIBENZOLAR-S-METHYL AND METHYL JASMONATE

Table A4.4.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated with MeJA, acibenzolar and MeJA + acibenzolar. Flowers were inoculated with 10^4 *B. cinerea* conidia L^{-1}.

Treatments	N		Mean Rank
Control	60		325.74
MeJA gas	60		143.25
MeJA pulse	60		278.45
MeJA spray	60		242.66
Acibenzolar	60		227.18
MeJA gas + Acibenzolar	60		177.21
MeJA pulse + Acibenzolar	60		279.69
MeJA spray + Acibenzolar	60		249.82
Total	480		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	7	76.012	.000

Table A4.4.2: Non-parametric test (Kruskal-Wallis) for disease severity of MeJA, acibenzolar and MeJA + acibenzolar treated freesia var. 'Cote d'Azur' incubated at 12, and 20°C.

Temperature (°C)	N		Mean Rank
12	240		218.96
20	240		262.04
Total	480		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	1	11.772	.001

Table A4.4.3: ANOVA table for disease severity of freesia var. 'var. 'Cote d'Azur' flowers treated with MeJA and MeJA + acibenzolar. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 12 and 20°C in the dark.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	57.879	15	3.859	7.482	.000
Intercept	1355.424	1	1355.424	2628.181	.000
CHEMICAL TREATMENT (C)	43.857	7	6.265	12.149	.000
TEMPERATURE (T)	9.577	1	9.577	18.569	.000
C * T	4.445	7	.635	1.231	.284
Error	239.297	464	.516		
Total	1652.600	480			
Corrected Total	297.176	479			

Table A4.4.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers with MeJA and MeJA + acibenzolar. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 12 and 20°C in the dark. Numbers on the same column are not significantly different at P = 0.05

Treatments	N	1	2	3	4	5	6	7	8
MeJA gas 20 [b]	30	1.0800							
MeJA gas + Acib 12	30	1.1733	1.1733						
MeJA gas 12	30	1.2300	1.2300						
Acibenzolar 12	30	1.4033	1.4033	1.4033					
MeJA gas + Acib 20	30	1.4367	1.4367	1.4367	1.4367				
MeJA spray 12	30		1.5567	1.5567	1.5567	1.5567			
MeJA pulse 12	30			1.6567	1.6567	1.6567	1.6567		
MeJA spray + Acib 12	30			1.6633	1.6633	1.6633	1.6633		
MeJA pulse + Acib 12	30			1.7200	1.7200	1.7200	1.7200		
MeJA spray 20	30			1.8100	1.8100	1.8100	1.8100	1.8100	
Acibenzolar 20	30			1.8233	1.8233	1.8233	1.8233	1.8233	
MeJA spray + Acib 20	30				1.8567	1.8567	1.8567	1.8567	
Control 12	30					1.9100	1.9100	1.9100	
MeJA pulse + Acib 20	30						2.0367	2.0367	2.0367
MeJA pulse 20	30							2.1567	2.1567
Control 20	30								2.3733
Sig.		.087	.065	.052	.052	.107	.081	.104	.086

[a] MeJA gas = 0.1 μM L^{-1}, MeJA pulse = 200 μM, MeJA spray = 600 μM, acibenzolar = 0.15 g AIL^{-1} and Control = 0 μM.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.4.5: ANOVA table for lesion number of freesia var. 'var. 'Cote d'Azur' flowers treated with MeJA and MeJA + acibenzolar. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	32209.392	15	2147.293	8.274	.000
Intercept	1062765.408	1	1062765.408	4095.258	.000
CHEMICAL TREATMENT (C)	26492.725	7	3784.675	14.584	.000
TEMPERATURE (T)	1695.008	1	1695.008	6.532	.011
C * T	4021.658	7	574.523	2.214	.032
Error	120413.200	464	259.511		
Total	1215388.000	480			
Corrected Total	152622.592	479			

Table A4.4.6: Lesion number means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers were treated with MeJA and MeJA + acibenzolar. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 12 and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4	5	6	7
MeJA gas 20 [b]	30	30.5000						
MeJA gas + Acib 20	30	36.0667	36.0667					
MeJA gas + Acib 12	30		41.5333	41.5333				
MeJA gas 12	30		41.9000	41.9000				
MeJA spray 20	30		42.8333	42.8333	42.8333			
MeJA spay + Acib 20	30		43.6333	43.6333	43.6333	43.6333		
MeJA pulse 20	30			46.2667	46.2667	46.2667		
Acibenzolar 20	30			46.4000	46.4000	46.4000		
MeJA spray 12	30			48.0667	48.0667	48.0667		
Acibenzolar 12	30			48.1333	48.1333	48.1333		
MeJA pulse + Acib 20	30			48.5000	48.5000	48.5000		
MeJA pulse 12	30			48.7000	48.7000	48.7000		
MeJA spray + Acib 12	30				52.3000	52.3000	52.3000	
MeJA pulse + Acib 12	30					53.0667	53.0667	
Control 12	30						57.7667	
Control 20	30							67.2000
Sig.		.181	.106	.156	.054	.055	.217	1.000

[a] MeJA gas = 0.1 μM L^{-1}, MeJA pulse = 200 μM, MeJA spray = 600 μM, acibenzolar = 0.15 g AIL^{-1} and Control = 0 μM.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.4.7: ANOVA table for lesion diameter of freesia var. 'var. 'Cote d'Azur' flowers treated with MeJA and MeJA + acibenzolar. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12.345	15	.823	25.746	.000
Intercept	233.704	1	233.704	7310.945	.000
CHEMICAL TREATMENT (C)	10.606	7	1.515	47.396	.000
TEMPERATURE (T)	1.231	1	1.231	38.515	.000
C * T	.508	7	7.264E-02	2.272	.028
Error	14.832	464	3.197E-02		
Total	260.882	480			
Corrected Total	27.178	479			

`able A4.4.8: Lesion diameter means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers were reated with MeJA and MeJA + acibenzolar. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 12 and 20°C. Numbers n the same column are not significantly different at P = 0.05

`reatments [a]	N	1	2	3	4	5	6	7	8	9
/leJA gas 12 [b]	30	.4627								
/leJA gas + Acib 12	30	.4803								
/leJA gas 20	30	.4827								
/leJA gas + Acib 20	30		.5873							
/leJA spray 12	30		.6227	.6227						
/leJA spray + Acib 12	30		.6333	.6333						
\cibenzolar 12	30		.6597	.6597	.6597					
/leJA spray 20	30	.6783	.6783	.6783	.6783					
/leJA pulse + Acib 12	30		.7030	.7030	.7030	.7030				
/leJA spay + Acib 20	30			.7383	.7383	.7383	.7383			
/leJA pulse 12	30			.7500	.7500	.7500	.7500			
\cibenzolar 20	30				.7720	.7720	.7720	.7720		
/leJA pulse + Acib 20	30					.7973	.7973	.7973		
/leJA pulse 20	30						.8117	.8117		
:ontrol 12	30							.8653		
:ontrol 20	30								1.1197	

;ig.	.687	.079	.123	.081	.070	.068	.161	.064	1.000

[a] MeJA gas = 0.1 μM L^{-1}, MeJA pulse = 200 μM, MeJA spray = 600 μM, acibenzolar = 0.15 g AIL^{-1} and Control = 0 μM.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A4.4.9: One-way ANOVA table for lesion diameter of detached petals treated var. with MeJA and MeJA + acibenzolar.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.774	7	1.825	7.391	.000
Within Groups	17.777	72	.247		
Total	30.550	79			

APPENDIX 4.5: EFFECTS OF POSTHARVEST UV-C IRRADIATION

4.5.1 Effect of UV-C irradiation on disease variables

Table A4.5.1.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers, irradiated with UV-C, inoculated with 10^4 *B. cinerea* conidia mL^{-1} before and after irradiation.

Treatments	N	Mean Rank
0 kJ m^{-2}	50	171.00
0.5 kJ m^{-2}	50	115.28
1 kJ m^{-2}	50	95.80
2.5 kJ m^{-2}	50	115.08
5 kJ m^{-2}	50	130.34
Total	250	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	3	30.728	.000

Table A4.5.1.2: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers, irradiated with UV-C, inoculated with 10^4 *B. cinerea* conidia mL^{-1} before and after irradiation.

Temperature (°C)	N	Mean Rank
Before UV-C	100	184.57
After UV-C	150	86.12
Total	250	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	1	112.078	.000

Table A4.5.1.3: ANOVA table for disease severity of freesia var. 'var. 'Cote d'Azur' flowers irradiated with UV-C. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} before and after UV-C irradiation and incubated at 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	280.901	9	31.211	69.285	.000
Intercept	1170.240	1	1170.240	2597.771	.000
CHEMICAL TREATMENT (C)	37.358	4	9.339	20.732	.000
INOCULATION (I)	177.848	1	177.848	394.798	.000
C * I	55.734	4	13.934	30.931	.000
Error	108.115	240	.450		
Total	1425.340	250			
Corrected Total	389.016	249			

Table A4.5.1.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers were irradiated with UV-C. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} before and after UV-C irradiation and incubated at 20°C.

Treatments [a]	N	1	2	3	4	5
2.5 a [b]	30	.7233				
1 a	30	.8000				
5 a	30	1.0800	1.0800			
0.5 a	30		1.4533			
0.5 b	20			2.3900		
1 b	20			2.6350	2.6350	
Control a	30			2.6800	2.6800	
Control b	20				3.0250	
2.5 b	20					3.5750
5 b	20					3.7200
Sig.		.082	.054	.159	.056	.454

[a] Control = 0 kJ m^{-2}, 0.5 = 0.5 kJ m^{-2}, 1 = 1kJ m^{-2}, 2.5 = 2.5 kJ m^{-2}, 5 = 5 kJ m^{-2}

[b] a = UV-C after *B. cinerea* inoculation, b = UV-C before *B. cinerea* inoculation.

Table A4.5.1.5: ANOVA table for lesion number of freesia var. 'var. 'Cote d'Azur' flowers irradiated with UV-C. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} before and after UV-C irradiation and incubated at 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	195890.003	9	21765.556	72.258	.000
Intercept	845666.304	1	845666.304	2807.458	.000
CHEMICAL TREATMENT (C)	26113.243	4	6528.311	21.673	.000
INOCULATION (I)	135033.216	1	135033.216	448.286	.000
C * I	31479.931	4	7869.983	26.127	.000
Error	72293.133	240	301.221		
Total	1013910.000	250			
Corrected Total	268183.136	249			

Table A4.5.1.6: Lesion number means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers were irradiated with UV-C. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} before and after UV-C irradiation and incubated at 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4
2.5 a [b]	30	20.9333			
1 a	30	25.3000			
5 a	30	26.2000			
0.5 a	30		41.0667		
0.5 b	20			61.9500	
Control a	30			64.7000	
1 b	20			68.6500	
Control b	20				89.9500
2.5 b	20				97.3500
5 b	20				97.5000
Sig.		.325	1.000	.209	.156

[a] Control = 0 kJ m^{-2}, 0.5 = 0.5 kJ m^{-2}, 1 = 1kJ m^{-2}, 2.5 = 2.5 kJ m^{-2}, 5 = 5 kJ m^{-2}

[b] a = UV-C after *B. cinerea* inoculation, b = UV-C before *B. cinerea* inoculation.

Table A4.5.1.7: ANOVA table for lesion diameter of freesia var. 'var. 'Cote d'Azur' flowers irradiated with UV-C. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} before and after UV-C irradiation and incubated at 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.568	9	.174	5.393	.000
Intercept	172.266	1	172.266	5333.556	.000
CHEMICAL TREATMENT (C)	.401	4	.100	3.104	.016
INOCULATION (I)	.849	1	.849	26.292	.000
C * I	.359	4	8.986E-02	2.782	.027
Error	7.752	240	3.230E-02		
Total	183.759	250			
Corrected Total	9.319	249			

Table 4.5.1.8: Lesion diameter means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers were irradiated with UV-C. The flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} before and after UV-C irradiation and incubated at 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4
1 a [b]	30	.7270			
2.5 a	30	.7660	.7660		
0.5 a	30	.7860	.7860		
0.5 b	20	.7865	.7865		
5 a	30	.8103	.8103	.8103	
Control a	30		.8493	.8493	
1 b	20			.9000	.9000
Control b	20			.9030	.9030
2.5 b	20				.9625
5 b	20				.9815
Sig.		.156	.156	.104	.154

[a] Control = 0 kJ m^{-2}, 0.5 = 0.5 kJ m^{-2}, 1 = 1kJ m^{-2}, 2.5 = 2.5 kJ m^{-2}, 5 = 5 kJ m^{-2}

[b] a = UV-C after *B. cinerea* inoculation, b = UV-C before *B. cinerea* inoculation.

Table A4.5.1.9: Parameters estimated for the linear model ($y = y_0 + ax + bx^2 + cx^3$) used to describe the effects of UV-C irradiation before or after artificial inoculation on disease severity, lesion number and lesion diameter on freesia petals.

UV-C irradiation	Estimated parameters				Coefficient (R^2)
	y_0	a	b	c	
a. Disease severity					
Before inoculation	2.7	– 2.97	1.21	0.135	0.99
After inoculation	2.95	– 1.26	0.94	0.132	0.98
b. Lesion number					
Before inoculation	65.23	– 61.13	24.07	2.68	0.99
After inoculation	87.58	– 54.16	35.36	4.82	0.93
c. Lesion diameter					
Before inoculation	0.85	– 0.197	0.092	0.011	0.97
After inoculation	0.88	– 0.098	0.084	0.012	0.69

4.5.2 Effect of UV-C irradiation on freesia vase life

Table A4.5.2.1: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	24	.000		
Total	.000	27			

Table A4.5.2.2: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 1).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.071E-02	4	1.518E-02	.464	.762
Within Groups	.982	30	3.274E-02		
Total	1.043	34			

Table A4.5.2.3: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.207	4	5.179E-02	1.012	.417
Within Groups	1.536	30	5.119E-02		
Total	1.743	34			

Table A4.5.2.4: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 3).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.561	4	.140	2.201	.093
Within Groups	1.911	30	6.369E-02		
Total	2.471	34			

Table A4.5.2.5: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.546	4	.387	1.503	.226
Within Groups	7.714	30	.257		
Total	9.261	34			

Table A4.5.2.6: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 5).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.543	4	.386	.970	.438
Within Groups	11.929	30	.398		
Total	13.471	34			

Table A4.5.2.7: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.204	4	.551	1.714	.173
Within Groups	9.643	30	.321		
Total	11.846	34			

Table A4.5.2.8: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 7).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.636	4	.409	2.948	.036
Within Groups	4.161	30	.139		
Total	5.796	34			

Table A4.5.2.9: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.589	4	.147	2.661	.052
Within Groups	1.661	30	5.536E-02		
Total	2.250	34			

Table A4.5.2.10: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 9).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.232	4	5.804E-02	2.438	.069
Within Groups	.714	30	2.381E-02		
Total	.946	34			

Table A4.5.2.11: One-way ANOVA table for wilt score of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 10).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.500E-02	4	6.250E-03	.955	.447
Within Groups	.196	30	6.548E-03		
Total	.221	34			

Table A4.5.2.12: One-way ANOVA table for flower fresh weight of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 0).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.	.
Within Groups	.000	16	.000		
Total	.000	19			

Table A4.5.2.13: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	37.695	4	9.424	2.066	.110
Within Groups	136.820	30	4.561		
Total	174.515	34			

Table A4.5.2.14: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 4).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	65.944	4	16.486	1.158	.349
Within Groups	427.115	30	14.237		
Total	493.059	34			

Table A4.5.2.15: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 6).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	245.797	4	61.449	1.760	.163
Within Groups	1047.293	30	34.910		
Total	1293.090	34			

Table A4.5.2.16: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 8).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	407.170	4	101.793	1.763	.162
Within Groups	1732.135	30	57.738		
Total	2139.305	34			

Table A4.5.2.17: One-way ANOVA table for flower relative fresh weight of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses (day 10).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	215.054	4	53.764	.586	.675
Within Groups	2751.854	30	91.728		
Total	2966.908	34			

Table A4.5.2.18: One-way ANOVA table for vase lives of freesia var. 'Cote d'Azur' flowers irradiated with UV-C at five doses.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.829	4	1.957	3.736	.014
Within Groups	15.714	30	.524		
Total	23.543	34			

APPENDIX 5: GLASSHOUSE TRIALS OF ACIBENZOLAR-S-METHYL, *AUREOBASIDIUM PULLULLANS* AND METHYL JASMONATE TO SUPPRESS SPECKING ON CUT FREESIA FLOWERS CAUSED BY *BOTRYTIS CINEREA*

APPENDIX 5.1: PREHARVEST ACIBENZOLAR-S-METHYL TREATMENTS

A5.1.1 Effect of preharvest acibenzolar-S-methyl treatment (glasshouse trial 2000)

Table A5.1.1.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2000. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C.

Treatments	N	Mean Rank
Control	16	102.00
Acibenzolar 0.15	16	78.50
Acibenzolar 0.3	16	42.72
Acibenzolar 0.6	16	57.75
Iprodione	16	66.09
Acibenzolar 0.15 + iprodione	16	49.34
Acibenzolar 0.3 + iprodione	16	56.34
Acibenzolar 0.6 + iprodione	16	63.25

Variable	df	Chi-square	Asymp. Sig.
Disease severity	7	31.210	.000

Table A5.1.1.2: ANOVA table for disease severity of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2000. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	77.867	7	11.124	6.073	.000
Within Groups	219.813	120	1.832		
Total	297.680	127			

Table A5.1.1.3: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2000. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatment [a]		1	2	3
Acibenzolar 1 + iprodione	16	1.0625		
Acibenzolar 2	16	1.1875		
Acibenzolar 2 + iprodione	16	1.4375		
Acibenzolar 3	16	1.6250	1.6250	
Acibenzolar 3 + iprodione	16	1.7500	1.7500	
Iprodione	16	2.0000	2.0000	
Acibenzolar 1	16		2.5000	
Control	16			3.6250
Sig.		.089	.098	1.000

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}

A5.1.2 Effect of preharvest acibenzolar-S-methyl treatment (glasshouse trial 2001)

Table A5.1.2.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N		Mean Rank
Control	120		331.39
Acibenzolar 1	120		292.38
Acibenzolar 2	120		303.47
Acibenzolar 3	120		332.69
Iprodione	120		242.57
Total	600		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	4	21.921	.000

Table A5.1.2.2: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N		Mean Rank
5	200		245.68
12	200		322.25
20	200		333.57
Total	600		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	30.811	.000

Table A5.1.2.3: ANOVA table for disease severity of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	74.258	14	5.304	5.534	.000
Intercept	1509.640	1	1509.640	1574.971	.000
CHEMICAL TREATMENT (C)	22.125	4	5.531	5.771	.000
TEMPERATURE (T)	31.106	2	15.553	16.226	.000
C * T	21.027	8	2.628	2.742	.006
Error	560.734	585	.959		
Total	2144.632	600			
Corrected Total	634.992	599			

Table A5.1.2.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Iprodione 5 [b]	40	.8725				
Acibenzolar 2 5	40	1.0331	1.0331			
Acibenzolar 1 5	40	1.1648	1.1648			
Iprodione 20	40	1.2940	1.2940	1.2940		
Acibenzolar 5	40		1.4616	1.4616	1.4616	
Acibenzolar 1 12	40		1.4861	1.4861	1.4861	
Acibenzolar 3 12	40			1.6515	1.6515	
Iprodione 12	40			1.6565	1.6565	
Control 20	40			1.7309	1.7309	1.7309
Acibenzolar 2 12	40				1.7961	1.7961
Control 5	40				1.8200	1.8200
Acibenzolar 1 20	40				1.8488	1.8488
Control 12	40				1.8585	1.8585
Acibenzolar 2 20	40				1.9319	1.9319
Acibenzolar 3 20	40					2.1871
Sig.		.078	.064	.081	.073	.072

[a] Acibenzolar 1, 2, 3 = 0.15 0.30 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.2.5: ANOVA table for lesion number of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	107285.040	14	7663.217	7.682	.000
Intercept	1406407.335	1	1406407.335	1409.838	.000
CHEMICAL TREATMENT (C)	27152.907	4	6788.227	6.805	.000
TEMPERATURE (T)	71214.010	2	35607.005	35.694	.000
C * T	8918.123	8	1114.765	1.117	.349
Error	583576.625	585	997.567		
Total	2097269.000	600			
Corrected Total	690861.665	599			

Table A5.1.2.6: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4
Iprodione 5 [b]	40	22.8750			
Acibenzolar 2 5	40	23.6750			
Acibenzolar 1 5	40	29.0750	29.0750		
Iprodione 20	40		41.1500	41.1500	
Control 5	40		42.3000	42.3000	
Acibenzolar 5	40			47.1250	47.1250
Iprodione 12	40			50.7000	50.7000
Acibenzolar 2 12	40			53.3500	53.3500
Acibenzolar 1 12	40			53.4750	53.4750
Acibenzolar 2 20	40			56.4000	56.4000
Acibenzolar 3 12	40				58.5500
Acibenzolar 3 20	40				60.7500
Acibenzolar 1 20	40				61.1750
Control	40				62.2250
Control 12	40				63.4000
Sig.		.412	.076	.062	.053

[a] Acibenzolar 1, 2, 3 = 0.15 0.30 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.2.7: ANOVA table for lesion diameter of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.161	14	.654	4.080	.000
Intercept	288.688	1	288.688	1800.180	.000
CHEMICAL TREATMENT (C)	4.103	4	1.026	6.397	.000
TEMPERATURE (T)	3.534	2	1.767	11.020	.000
C * T	1.524	8	.190	1.188	.305
Error	69.759	435	.160		
Total	367.609	450			
Corrected Total	78.920	449			

Table A5.1.2.8: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Acibenzolar 1 5 [b]	30	29.6333				
Control 5	30	30.9000				
Acibenzolar 5	30	31.4667				
Iprodione 5	30	32.1000				
Control 12	30	34.6333	34.6333			
Acibenzolar 2 5	30	34.7333	34.7333			
Control	30	41.5333	41.5333			
Acibenzolar 2 12	30	46.2667	46.2667	46.2667		
Acibenzolar 3 12	30	48.5667	48.5667	48.5667	48.5667	
Iprodione 12	30		52.0667	52.0667	52.0667	
Iprodione 20	30		52.8333	52.8333	52.8333	
Acibenzolar 1 20	30			60.5333	60.5333	60.5333
Acibenzolar 2 20	30			62.9667	62.9667	62.9667
Acibenzolar 3 20	30				67.1000	67.1000
Acibenzolar 1 12	30					75.6333
Sig.		.054	.059	.080	.050	.099

[a] Acibenzolar 1, 2, 3 = 0.15 0.30 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.2.9: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N		Mean Rank
Control	88		324.99
Acibenzolar 0.15	88		214.80
Acibenzolar 0.30	88		177.63
Acibenzolar 0.60	88		196.16
Iprodione	88		188.91
Total	440		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	4	79.417	.000

Table A5.1.2.10: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N		Mean Rank
5	160		143.05
12	160		258.23
20	120		273.46
Total	440		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	95.661	.000

Table A5.1.2.11: ANOVA table for disease severity of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	156.779	14	11.198	22.222	.000
Intercept	3071.808	1	3071.808	6095.724	.000
CHEMICAL TREATMENT (C)	52.642	4	13.160	26.116	.000
TEMPERATURE (T)	86.743	2	43.371	86.067	.000
C * T	13.814	8	1.727	3.427	.001
Error	214.170	425	.504		
Total	3424.702	440			
Corrected Total	370.948	439			

Table A5.1.2.12: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5	6	7
Acibenzolar 2 5 [b]	32	1.6297						
Iprodione 5	32	1.7928						
Acibenzolar 3 5	32	1.8278						
Acibenzolar 1 5	32	1.9288						
Iprodione 20	24		2.4792					
Acibenzolar 2 12	32		2.6678	2.6678				
Acibenzolar 3 12	32		2.7159	2.7159	2.7159			
Acibenzolar 1 12	32		2.8025	2.8025	2.8025	2.8025		
Acibenzolar 2 20	24			2.9150	2.9150	2.9150		
Iprodione 12	32			2.9703	2.9703	2.9703		
Control 5	32			3.0656	3.0656	3.0656		
Acibenzolar 3 20	24				3.0917	3.0917	3.0917	
Acibenzolar 1 20	24					3.1425	3.1425	3.1425
Control 20	24						3.4717	3.4717
Control 12	32							3.4975
Sig.		.147	.116	.062	.079	.114	.054	.072

[a] Acibenzolar 1, 2, 3 = 0.15 0.30 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.2.13: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	169588.580	14	12113.470	18.110	.000
Intercept	2290623.01	1	2290623.01	3424.48	.000
CHEMICAL TREATMENT (C)	76590.945	4	19147.736	28.626	.000
TEMPERATURE (T)	75141.305	2	37570.653	56.168	.000
C * T	12848.457	8	1606.057	2.401	.015
Error	284280.635	425	668.896		
Total	2825717.00	440			
Corrected Total	453869.216	439			

Table A5.1.2.14: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5	6	7	8	9
Acibenzolar 2	5 [b]	32	47.9375							
Iprodione	5	32	51.5313							
Acibenzolar 1	5	32	54.4063	54.4063						
Acibenzolar 3	20	24	55.5417	55.5417						
Iprodione	20	24	57.9167	57.9167	57.9167					
Acibenzolar 2	20	24	59.7083	59.7083	59.7083	59.7083				
Acibenzolar 3	5	32		67.4063	67.4063	67.4063	67.4063			
Acibenzolar 1	20	24			71.3333	71.3333	71.3333	71.3333		
Acibenzolar 2	12	32				73.1250	73.1250	73.1250	73.1250	
Acibenzolar 1	12	32					81.4063	81.4063	81.4063	81.4063
Acibenzolar 3	12	32						84.7500	84.7500	84.7500
Control	20	24							86.4167	86.4167
Control	5	32							87.3125	87.3125
Iprodione	12	32								91.2813
Control	12	32								122.1875
Sig.			.134	.090	.072	.072	.060	.072	.063	.203	1.000

[a] Acibenzolar 1, 2, 3 = 0.15 0.30 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.2.15: ANOVA table for lesion diameter of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.196	14	.514	6.706	.000
Intercept	417.986	1	417.986	5453.996	.000
CHEMICAL TREATMENT (C)	3.622	4	.905	11.815	.000
TEMPERATURE (T)	3.042	2	1.521	19.845	.000
C * T	.427	8	5.339E-02	.697	.695
Error	41.002	535	7.664E-02		
Total	474.804	550			
Corrected Total	48.197	549			

Table A5.1.2.16: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Acibenzolar 2 5 [b]	40	.7095				
Acibenzolar 1 5	40	.7137				
Acibenzolar 2 20	30	.7640	.7640			
Acibenzolar 3 5	40	.7870	.7870	.7870		
Acibenzolar 1 20	30	.8310	.8310	.8310	.8310	
Iprodione 5	40		.8772	.8772	.8772	
Acibenzolar 3 20	30		.8797	.8797	.8797	
Acibenzolar 2 12	40		.8808	.8808	.8808	
Acibenzolar 1 12	40		.8902	.8902	.8902	
Iprodione 20	20		.8985	.8985	.8985	
Control 5	40		.9022	.9022	.9022	
Acibenzolar 3 12	40			.9188	.9188	
Control 20	40				.9565	.9565
Iprodione 12	40					1.0783
Control 12	40					1.0903
Sig.		.103	.079	.095	.113	.055

[a] Acibenzolar 1, 2, 3 = 0.15 0.30 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.2.17: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N	Mean Rank
Control	110	297.30
Acibenzolar 0.15	110	270.48
Acibenzolar 0.30	110	294.40
Acibenzolar 0.60	110	303.85
Iprodione	110	211.46
Total	550	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	4	25.575	.000

Table A5.1.2.18: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N	Mean Rank
5	200	244.15
12	200	322.23
20	150	254.99
Total	550	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	28.095	.000

Table A5.1.2.19: ANOVA table for disease severity of freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	32.719	14	2.337	5.122	.000
Intercept	585.938	1	585.938	1284.236	.000
CHEMICAL TREATMENT (C)	12.656	4	3.164	6.935	.000
TEMPERATURE (T)	14.383	2	7.191	15.762	.000
C * T	4.360	8	.545	1.195	.300
Error	244.096	535	.456		
Total	883.190	550			
Corrected Total	276.815	549			

Table A5.1.2.20: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Numbers within the same column are not significantly different at P = 0.05. Freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Iprodione 5 [b]	40	.6325				
Iprodione 20	30	.7567	.7567			
Acibenzolar 1 5	40	.7950	.7950			
Acibenzolar 2 20	30	.8667	.8667	.8667		
Iprodione 12	40	.8775	.8775	.8775		
Control 20	30	.9533	.9533	.9533		
Acibenzolar 3 5	40		1.0000	1.0000	1.0000	
Control 5	40		1.0250	1.0250	1.0250	
Acibenzolar 3 20	30		1.0767	1.0767	1.0767	
Acibenzolar 1 20	30		1.0967	1.0967	1.0967	
Acibenzolar 2 5	40		1.1075	1.1075	1.1075	
Acibenzolar 1 12	40			1.1725	1.1725	1.1725
Acibenzolar 2 12	40				1.3250	1.3250
Control 12	40					1.4625
Acibenzolar 3 12	40					1.4775
Sig.		.078	.065	.108	.079	.080

[a] Acibenzolar 1, 2, 3 = 0.15 0.30 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.2.21: ANOVA table for lesion number of freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	141653.436	14	10118.103	11.374	.000
Intercept	1915199.46	1	1915199.46	2152.93	.000
CHEMICAL TREATMENT (C)	38809.014	4	9702.254	10.907	.000
TEMPERATURE (T)	86034.823	2	43017.411	48.357	.000
C * T	12479.802	8	1559.975	1.754	.084
Error	475922.775	535	889.575		
Total	2618270.00	550			
Corrected Total	617576.211	549			

Table A5.1.2.22: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5	6	7	8
Iprodione 5 [b]	40	36.2500							
Acibenzolar 3 20	30	44.6667	44.6667						
Acibenzolar 1 5	40	45.4250	45.4250	45.4250					
Acibenzolar 1 20	30	45.7000	45.7000	45.7000					
Acibenzolar 3 5	40	47.8000	47.8000	47.8000	47.8000				
Acibenzolar 2 20	30	49.1000	49.1000	49.1000	49.1000				
Acibenzolar 20	30		53.7333	53.7333	53.7333	53.7333			
Acibenzolar 2 5	40		58.2500	58.2500	58.2500	58.2500			
Iprodione 12	40			60.8250	60.8250	60.8250			
Control 20	30				62.8333	62.8333			
Control 5	40					65.2750	65.2750		
Acibenzolar 1 12	40					66.8000	66.8000		
Acibenzolar 2 12	40						79.7250	79.7250	
Acibenzolar 3 12	40							82.3000	82.3000
Control 12	40								94.6250
Sig.		.112	.097	.058	.060	.106	.051	.714	.080

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.2.23: ANOVA table for lesion diameter of freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.942	14	.210	4.943	.000
Intercept	158.571	1	158.571	3729.98	.000
CHEMICAL TREATMENT (C)	1.548	4	.387	9.104	.000
TEMPERATURE (T)	.257	2	.128	3.018	.050
C * T	.883	8	.110	2.597	.009
Error	20.619	485	4.251E-02		
Total	183.569	500			
Corrected Total	23.560	499			

Table A5.1.2.24: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5	6
Acibenzolar 1 12 [b]	40	.4473					
Acibenzolar 3 5	30	.4657	.4657				
Acibenzolar 3 12	40	.4978	.4978	.4978			
Acibenzolar 2 12	40	.5183	.5183	.5183	.5183		
Iprodione 12	40	.5193	.5193	.5193	.5193		
Acibenzolar 1 20	30	.5467	.5467	.5467	.5467		
Acibenzolar 2 20	30	.5500	.5500	.5500	.5500		
Acibenzolar 1 5	30	.5637	.5637	.5637	.5637		
Iprodione 20	30		.5807	.5807	.5807	.5807	
Acibenzolar 2 5	30			.5847	.5847	.5847	
Iprodione 5	30			.6087	.6087	.6087	
Control 5	30			.6100	.6100	.6100	
Acibenzolar 3 20	30				.6180	.6180	
Control 20	30					.6877	.6877
Control 12	40						.7270
Sig.		.050	.053	.065	.104	.066	.440

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

A5.1.3 Effect of preharvest acibenzolar-S-methyl treatment (glasshouse trial 2002)

Table A5.1.3.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N	Mean Rank
Control	90	238.26
Acibenzolar 1	90	227.49
Acibenzolar 2	90	238.13
Acibenzolar 3	90	226.86
Iprodione	90	196.77
Total	450	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	4	7.000	0.136

Table A5.1.3.2: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N		Mean Rank
5	150		144.14
12	150		288.24
20	150		244.12
Total	450		
Variable	**df**	**Chi-square**	**Asymp. Sig.**
Disease severity	2	110.300	.000

Table A5.1.3.3: ANOVA table for disease severity of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	38.875	14	2.777	8.127	.000
Intercept	104.257	1	104.257	305.136	.000
CHEMICAL TREATMENT (C)	2.464	4	.616	1.803	.127
TEMPERATURE (T)	33.410	2	16.705	48.891	.000
C * T	3.001	8	.375	1.098	.363
Error	148.628	435	.342		
Total	291.760	450			
Corrected Total	187.503	449			

Table A5.1.3.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatment [a]		1	2	3	4	5
Control 5 [b]	30	2.000E-02				
Acibenzolar 1 5	30	8.667E-02				
Iprodione 5	30	9.000E-02				
Acibenzolar 3 5	30	.1567	.1567			
Acibenzolar 2 5	30	.1800	.1800			
Iprodione 20	30		.4333	.4333		
Acibenzolar 1 20	30		.4733	.4733	.4733	
Acibenzolar 3 20	30			.5333	.5333	.5333
Iprodione 12	30			.5733	.5733	.5733
Acibenzolar 2 20	30			.6400	.6400	.6400
Acibenzolar 3 12	30			.7500	.7500	.7500
Acibenzolar 1 12	30			.7733	.7733	.7733
Acibenzolar 2 12	30				.7967	.7967
Control 12	30					.8400
Control 20	30					.8733
Sig.		.355	.054	.051	.064	.054

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.3.5: ANOVA table for lesion number of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	59042.658	14	4217.333	6.071	.000
Intercept	173971.342	1	173971.342	250.429	.000
CHEMICAL TREATMENT (C)	4913.880	4	1228.470	1.768	.134
TEMPERATURE (T)	46135.058	2	23067.529	33.205	.000
C * T	7993.720	8	999.215	1.438	.178
Error	302192.000	435	694.694		
Total	535206.000	450			
Corrected Total	361234.658	449			

Table A5.1.3.6: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Control 5 [b]	30	2.2333				
Iprodione 5	30	3.4333				
Acibenzolar 1 5	30	4.4000				
Acibenzolar 3 5	30	7.1333	7.1333			
Acibenzolar 2 5	30	9.5333	9.5333			
Acibenzolar 1 20	30	17.0333	17.0333	17.0333		
Acibenzolar 3 12	30		20.5667	20.5667	20.5667	
Acibenzolar 3 20	30		21.1333	21.1333	21.1333	
Acibenzolar 1 12	30			25.1000	25.1000	25.1000
Iprodione 20	30			26.8000	26.8000	26.8000
Control 12	30			26.9667	26.9667	26.9667
Acibenzolar 2 20	30			27.5667	27.5667	27.5667
Iprodione 12	30			29.8333	29.8333	29.8333
Acibenzolar 2 12	30				33.0667	33.0667
Control 20	30					40.1333
Sig.		.056	.066	.112	.121	.056

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.3.7: ANOVA table for lesion diameter of freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.184	14	8.455E-02	5.556	.000
Intercept	80.955	1	80.955	5319.97	.000
CHEMICAL TREATMENT (C)	.167	4	4.167E-02	2.739	.029
TEMPERATURE (T)	.113	2	5.637E-02	3.704	.026
C * T	.794	8	9.923E-02	6.521	.000
Error	3.576	235	1.522E-02		
Total	96.836	250			
Corrected Total	4.760	249			

Table A5.1.3.8: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5	6	7
Acibenzolar 2 20 [b]	20	.4600						
Control 5	10	.5220	.5220					
Iprodione 5	10	.5350	.5350	.5350				
Acibenzolar 1 5	10		.5580	.5580	.5580			
Acibenzolar 2 5	10		.5650	.5650	.5650	.5650		
Acibenzolar 1 20	20		.5655	.5655	.5655	.5655		
Acibenzolar 3 12	20		.5880	.5880	.5880	.5880		
Control 12	20		.5970	.5970	.5970	.5970	.5970	
Acibenzolar 2 12	20		.6205	.6205	.6205	.6205	.6205	
Acibenzolar 3 20	20			.6280	.6280	.6280	.6280	.6280
Iprodione 12	20			.6300	.6300	.6300	.6300	.6300
Acibenzolar 3 5	10				.6430	.6430	.6430	.6430
Acibenzolar 1 12	20					.6630	.6630	.6630
Iprodione 20	20						.6965	.6965
Control 20	20							.7260
Sig.		.116	.062	.076	.115	.066	.056	.056

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.3.9: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N	Mean Rank
Control	60	171.98
Acibenzolar 0.15	60	131.67
Acibenzolar 0.30	60	145.48
Acibenzolar 0.60	60	148.16
Iprodione	60	155.22
Total	300	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	4	7.028	.134

Table A5.1.3.10: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. **'Cote** d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione **during** acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* **conidia** mL^{-1}.

Temperature	N	Mean Rank
5	100	64.21
12	100	194.60
20	100	192.68
Total	300	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	150.603	.000

Table A5.1.3.11: ANOVA table for disease severity of freesia var. 'Cote d'Azur' flowers **treated** with acibenzolar-S-methyl at three rates and iprodione during acibenzolar **glasshouse** trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and **incubated** at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	217.422	14	15.530	31.570	.000
Intercept	881.339	1	881.339	1791.607	.000
CHEMICAL TREATMENT (C)	7.259	4	1.815	3.689	.006
TEMPERATURE (T)	197.443	2	98.721	200.683	.000
C * T	12.721	8	1.590	3.232	.002
Error	140.199	285	.492		
Total	1238.960	300			
Corrected Total	357.621	299			

Table A5.1.3.12: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Acibenzolar 1 5 [b]	20	.4850				
Acibenzolar 2 5	20	.5350				
Control 5	20	.5750				
Iprodione 5	20	.5750				
Acibenzolar 3 5	20	.6650				
Acibenzolar 1 20	20		1.7050			
Iprodione 12	20		2.0050	2.0050		
Acibenzolar 2 20	20		2.0550	2.0550		
Acibenzolar 3 20	20		2.1100	2.1100	2.1100	
Acibenzolar 2 12	20			2.3100	2.3100	2.3100
Acibenzolar 1 12	20			2.3200	2.3200	2.3200
Acibenzolar 3 12	20			2.3300	2.3300	2.3300
Control 12	20				2.5850	2.5850
Iprodione 20	20					2.6900
Control 20	20					2.7650
Sig.		.481	.096	.207	.055	.072

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.3.13: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	272686.987	14	19477.642	44.411	.000
Intercept	887046.563	1	887046.563	2022.54	.000
CHEMICAL TREATMENT (C)	8076.987	4	2019.247	4.604	.001
TEMPERATURE (T)	252302.747	2	126151.373	287.636	.000
C * T	12307.253	8	1538.407	3.508	.001
Error	124995.450	285	438.581		
Total	1284729.00	300			
Corrected Total	397682.437	299			

Table A5.1.3.14: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Acibenzolar 1 5 [b]	20	11.0000				
Iprodione 5	20	13.1000				
Acibenzolar 3 5	20	14.0500				
Control 5	20	14.3000				
Acibenzolar 2 5	20	15.2000				
Acibenzolar 1 20	20		56.5000			
Iprodione 12	20		65.1000	65.1000		
Acibenzolar 2 12	20		68.5000	68.5000		
Acibenzolar 1 12	20			71.4000		
Acibenzolar 2 20	20			73.6000		
Acibenzolar 3 20	20			75.6000	75.6000	
Acibenzolar 3 12	20			75.7000	75.7000	
Control 12	20			77.3500	77.3500	
Iprodione 20	20				88.9000	88.9000
Control 20	20					95.3500
Sig.		.582	.087	.113	.066	.330

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.3.15: ANOVA table for lesion diameter of freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.696	14	.193	7.536	.000
Intercept	167.793	1	167.793	6565.49	.000
CHEMICAL TREATMENT (C)	1.889	4	.472	18.475	.000
TEMPERATURE (T)	2.393E-02	2	1.197E-02	.468	.627
C * T	.402	8	5.031E-02	1.968	.051
Error	6.006	235	2.556E-02		
Total	194.032	250			
Corrected Total	8.702	249			

Table A5.1.3.16: Lesion diameter means separated using Duncan's multiple range test at **P** = 0.05. Freesia var. 'Cote d'Azur' flowers treated with acibenzolar-S-methyl at three **rates** and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated **with** 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same **column** are not significantly different at P = 0.05

Treatments [a]		1	2	3
Acibenzolar 2 20 [b]	20	.7365		
Acibenzolar 3 12	20	.7470		
Acibenzolar 1 20	20	.7525		
Acibenzolar 1 12	20	.7585		
Acibenzolar 3 5	10	.8220	.8220	
Control 5	10	.8250	.8250	
Acibenzolar 2 12	20	.8295	.8295	
Acibenzolar 3 20	20	.8320	.8320	
Acibenzolar 2 5	10	.8350	.8350	
Acibenzolar 1 5	10	.8480	.8480	
Control 12	20		.9405	.9405
Control 20	20		.9500	.9500
Iprodione 20	20			.9810
Iprodione 12	20			1.0440
Iprodione 5	10			1.0520
Sig.		.114	.061	.089

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.3.17: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. **'Dukaat'** flowers treated with acibenzolar-S-methyl at three rates and iprodione during **acibenzolar** glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia **mL^{-1}**.

Treatments	N	Mean Rank
Control	90	265.48
Acibenzolar 0.15	90	189.87
Acibenzolar 0.30	90	199.36
Acibenzolar 0.60	90	225.26
Iprodione	90	247.54
Total	450	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	4	21.985	.000

Table A5.1.3.18: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N	Mean Rank
5	150	154.74
12	150	313.69
20	150	208.07
Total	450	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	118.780	.000

Table A5.1.3.19: ANOVA table for disease severity of freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	76.191	14	5.442	15.143	.000
Intercept	360.551	1	360.551	1003.208	.000
CHEMICAL TREATMENT (C)	10.827	4	2.707	7.531	.000
TEMPERATURE (T)	62.227	2	31.114	86.572	.000
C * T	3.137	8	.392	1.091	.368
Error	156.338	435	.359		
Total	593.080	450			
Corrected Total	232.529	449			

Table A5.1.3.20: Disease severity means separated according to Duncan's multiple range **test** at P = 0.05. Freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three **rates** and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated **with** 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same **column** are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5	6
Acibenzolar 1 5	[b] 30	.3967					
Acibenzolar 2 5	30	.4367					
Acibenzolar 3 5	30	.4533					
Acibenzolar 1 20	30	.4533					
Acibenzolar 2 20	30	.4767					
Iprodione 5	30	.5600					
Control 5	30	.7133	.7133				
Acibenzolar 3 20	30		.8933	.8933			
Iprodione 20	30		.9733	.9733	.9733		
Control 20	30			1.0767	1.0767	1.0767	
Acibenzolar 1 12	30				1.2433	1.2433	1.2433
Acibenzolar 3 12	30					1.3467	1.3467
Acibenzolar 2 12	30					1.3500	1.3500
Iprodione 12	30						1.5000
Control 12	30						1.5533
Sig.		.078	.113	.267	.099	.108	.074

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.3.21: ANOVA table for lesion number of freesia var. 'Dukaat' flowers treated **with** acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial **2002**. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, **and** 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	126076.733	14	9005.481	17.522	.000
Intercept	501000.500	1	501000.500	974.806	.000
CHEMICAL TREATMENT (C)	13800.378	4	3450.094	6.713	.000
TEMPERATURE (T)	108168.013	2	54084.007	105.232	.000
C * T	4108.342	8	513.543	.999	.436
Error	223567.767	435	513.949		
Total	850645.000	450			
Corrected Total	349644.500	449			

Table A5.1.3.22: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
Acibenzolar 1 5 [b]	30	14.1000				
Acibenzolar 2 5	30	15.5000				
Acibenzolar 1 20	30	17.0333				
Acibenzolar 2 20	30	17.6333				
Iprodione 5	30	19.3667	19.3667			
Acibenzolar 3 5	30	19.6667	19.6667			
Control 5	30	25.7000	25.7000	25.7000		
Iprodione 20	30		30.7333	30.7333		
Acibenzolar 3 20	30		31.0667	31.0667		
Control 20	30			35.4000		
Acibenzolar 3 12	30				47.4333	
Acibenzolar 1 12	30				48.1000	
Acibenzolar 2 12	30				54.0667	54.0667
Iprodione 12	30				59.5667	59.5667
Control 12	30					65.1333
Sig.		.088	.075	.132	.057	.073

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.1.3.23: ANOVA table for lesion diameter of freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.470	14	3.360E-02	4.057	.000
Intercept	65.631	1	65.631	7924.29	.000
CHEMICAL TREATMENT (C)	.254	4	6.354E-02	7.671	.000
TEMPERATURE (T)	1.244E-02	2	6.221E-03	.751	.474
C * T	.204	8	2.547E-02	3.076	.003
Error	1.118	135	8.282E-03		
Total	67.219	150			
Corrected Total	1.588	149			

Table A5.1.3.24: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with acibenzolar-S-methyl at three rates and iprodione during acibenzolar glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	N	1	2	3	4	5
Acibenzolar 1 20 [b]	10	.5160				
Acibenzolar 1 12	10	.5910	.5910			
Iprodione 5	10		.6340	.6340		
Acibenzolar 3 5	10		.6350	.6350		
Acibenzolar 1 5	10		.6470	.6470	.6470	
Control 12	10		.6500	.6500	.6500	.6500
Control 5	10		.6560	.6560	.6560	.6560
Iprodione 12	10		.6630	.6630	.6630	.6630
Control 20	10		.6640	.6640	.6640	.6640
Acibenzolar 2 5	10		.6740	.6740	.6740	.6740
Acibenzolar 3 20	10			.6900	.6900	.6900
Acibenzolar 2 12	10			.6920	.6920	.6920
Acibenzolar 3 12	10			.7250	.7250	.7250
Iprodione 20	10				.7410	.7410
Acibenzolar 2 20	10					.7440
Sig.		.065	.087	.063	.052	.052

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.
[b] 5, 12, 20 = 5, 12, and 20°C.

A5.1.4 PAL activity of acibenzolar treated flowers

Table A5.1.4.1: PAL activity means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' were treated with acibenzolar-S-methyl at three rates and incubated at 12°C (0 h). Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1
Control	9	72.0887
Acibenzolar 1	9	103.5181
Acibenzolar 3	9	117.5925
Acibenzolar 2	9	142.0043
Sig.		.278

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.

Table A5.1.4.2: PAL activity means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' were treated with acibenzolar-S-methyl at three rates and incubated at 12°C (6 h). Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2
Control	9	45.4719	
Acibenzolar 1	9	45.5670	
Acibenzolar 3	9	88.4798	88.4798
Acibenzolar 2	9		110.0480
Sig.		.168	.459

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.

Table A5.1.4.3: PAL activity means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' were treated with acibenzolar-S-methyl at three rates and incubated at 12°C (12 h). Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2
Acibenzolar 2	9	3.0534	
Acibenzolar 1	9	17.8616	
Acibenzolar 3	6	61.1762	
Control	9		231.0592
Sig.		.219	1.000

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.

Table A5.1.4.4: PAL activity means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' were treated with acibenzolar-S-methyl at three rates and incubated at 12°C (24 h). Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2
Acibenzolar 3	9	1.7006	
Acibenzolar 1	9	27.1734	
Acibenzolar 2	9		103.4802
Control	9		119.5658
Sig.		.374	.573

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.

Table A5.1.4.5: PAL activity means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' were treated with acibenzolar-S-methyl at three rates and incubated at 20°C (0 h). Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1
Control	9	72.0887
Acibenzolar 1	9	103.5181
Acibenzolar 3	9	117.5925
Acibenzolar 2	9	142.0043
Sig.		.255

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.

Table A5.1.4.6: PAL activity means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' were treated with acibenzolar-S-methyl at three rates and incubated at 20°C (6 h). Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2
Acibenzolar 3	9	45.5396	
Control	9	58.3637	
Acibenzolar 1	9	81.8438	81.8438
Acibenzolar 2	9	124.0532	124.0532
Sig.		.058	.066

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.

Table A5.1.4.7: PAL activity means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' were treated with acibenzolar-S-methyl at three rates and incubated at 20°C (12 h). Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1
Acibenzolar 3	9	76.4093
Acibenzolar 2	9	127.1907
Control	9	127.3628
Acibenzolar 1	9	161.3370
Sig.		.249

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.

Table A5.1.4.8: PAL activity means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' were treated with acibenzolar-S-methyl at three rates and incubated at 20°C (24 h). Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2
Acibenzolar 2	9	38.1905	
Acibenzolar 1	9	55.6140	55.6140
Control	9	83.1014	83.1014
Acibenzolar 3	9		95.6616
Sig.		.077	.071

[a] Acibenzolar 1, 2, 3 = 0.15, 0.30, 0.60 g AIL^{-1}, respectively. Control = 0 g AIL^{-1}.

APPENDIX 5.2: *AUREOBASIDIUM PULLULLANS* PREHARVEST TREATMENTS

A5.2.1 Effect of preharvest *Aureobasidium pullulans* treatment (glasshouse trial 2001)

Table A5.2.1.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cinderella' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N		Mean Rank
Control	120		132.93
A. pullulans	120		108.07
Total	240		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	1	7.781	.005

Table A5.2.1.2: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cinderella' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N		Mean Rank
5	80		106.74
12	80		135.11
20	80		119.65
Total	240		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	6.782	.034

Table A5.2.1.3: ANOVA table for disease severity of freesia var. 'Cinderella' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	18.238	5	3.648	3.403	.005
Intercept	623.342	1	623.342	581.597	.000
CHEMICAL TREATMENT (C)	8.805	1	8.805	8.215	.005
TEMPERATURE (T)	5.051	2	2.525	2.356	.097
C * T	4.383	2	2.191	2.045	.132
Error	250.795	234	1.072		
Total	892.375	240			
Corrected Total	269.034	239			

Table A5.2.1.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments		1	2
A. pullulans 5 [a]	40	1.0639	
A. pullulans 20	40	1.4621	1.4621
Control 20	40		1.7309
A. pullulans 12	40		1.7342
Control 5	40		1.8200
Control 12	40		1.8585
Sig.		.085	.130

[a] 5, 12, 20 = 5, 12, and 20°C.

Table A5.2.1.5: ANOVA table for lesion number of freesia var. 'Cinderella' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	39691.183	5	7938.237	5.531	.000
Intercept	611656.067	1	611656.067	426.182	.000
CHEMICAL TREATMENT (C)	7238.017	1	7238.017	5.043	.026
TEMPERATURE (T)	29617.758	2	14808.879	10.318	.000
C * T	2835.408	2	1417.704	.988	.374
Error	335836.750	234	1435.200		

Total	987184.000	240
Corrected Total	375527.933	239

Table A5.2.1.6: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments		1	2
A. pullulans 5 [a]	40	29.1750	
Control 5	40	42.3000	
A. pullulans 20	40	44.1000	
A. pullulans 12	40		61.7000
Control 20	40		62.2250
Control 12	40		63.4000
Sig.		.096	.852

[a] 5, 12, 20 = 5, 12, and 20°C.

Table A5.2.1.7: ANOVA table for lesion diameter of freesia var. 'Cinderella' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.832	5	.166	3.110	.010
Intercept	81.264	1	81.264	1518.38	.000
CHEMICAL TREATMENT (C)	.196	1	.196	3.660	.057
TEMPERATURE (T)	.422	2	.211	3.946	.021
C * T	.165	2	8.248E-02	1.541	.217
Error	10.383	194	5.352E-02		
Total	94.446	200			
Corrected Total	11.215	199			

Table A5.2.1.8: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cinderella' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments	N	1	2	3
A. pullulans 5 [a]	30	.5450		
A. pullulans 12	40	.5955	.5955	
Control 5	30	.6100	.6100	.6100
Control 20	30		.6877	.6877
A. pullulans 20	30		.6947	.6947
Control 12	40			.7270
Sig.		.287	.115	.061

[a] 5, 12, 20 = 5, 12, and 20°C.

Table A5.2.1.9: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N		Mean Rank
Control	88		99.52
A. pullulans	88		77.48
Total	176		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	1	8.601	.003

Table A5.2.1.10: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N		Mean Rank
5	64		71.55
12	64		101.55
20	48		93.70
Total	176		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	12.333	.002

Table A5.2.1.11: ANOVA table for disease severity of freesia var. 'Cote d'Azur' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.072	5	.414	7.271	.000
Intercept	549.071	1	549.071	9633.525	.000
CHEMICAL TREATMENT (C)	.491	1	.491	8.618	.004
TEMPERATURE (T)	1.428	2	.714	12.525	.000
C * T	.138	2	6.907E-02	1.212	.300
Error	9.689	170	5.700E-02		
Total	568.546	176			
Corrected Total	11.761	175			

Table A5.2.1.12: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatment	N	1	2
A. pullulans 5 [a]	32	1.5731	
Control 5	32		1.7488
A. pullulans 20	24		1.7758
A. pullulans 12	32		1.8388
Control 20	24		1.8754
Control 12	32		1.8834
Sig.		1.000	.055

[a] 5, 12, 20 = 5, 12, and 20°C.

Table A5.2.1.13: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	63487.563	5	12697.513	16.483	.000
Intercept	1456238.09	1	1456238.09	1890.40	.000
CHEMICAL TREATMENT (C)	8007.417	1	8007.417	10.395	.002
TEMPERATURE (T)	53361.673	2	26680.836	34.636	.000
C * T	1233.839	2	616.920	.801	.451
Error	130956.385	170	770.332		
Total	1703861.00	176			
Corrected Total	194443.949	175			

Table A5.2.1.14: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments		1	2	3
A. pullulans 5 [a]	32	66.8750		
A. pullulans 20	24	79.4167	79.4167	
Control 20	24		86.4167	
Control 5	32		87.2188	
A. pullulans 12	32			108.6875
Control 12	32			122.1875
Sig.		.086	.318	.065

[a] 5, 12, 20 = 5, 12, and 20°C.

Table A5.2.1.15: ANOVA table for lesion diameter of freesia var. 'Cote d'Azur' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.603	5	.321	3.689	.003
Intercept	196.906	1	196.906	2264.99	.000
CHEMICAL TREATMENT (C)	.671	1	.671	7.715	.006
TEMPERATURE (T)	.817	2	.409	4.700	.010
C * T	.106	2	5.315E-02	.611	.544
Error	18.604	214	8.693E-02		
Total	219.728	220			
Corrected Total	20.207	219			

Table A5.2.1.16: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments	N	1	2	3
A. pullulans 5 [a]	40	.8438		
Control 5	40	.9022	.9022	
A. pullulans 20	30	.9247	.9247	
A. pullulans 12	40	.9288	.9288	
Control 20	30		1.0390	1.0390
Control 12	40			1.0903
Sig.		.271	.072	.461

[a] 5, 12, 20 = 5, 12, and 20°C.

Table A5.2.1.17: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Dukaat' flowers treated *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001 and inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N		Mean Rank
Control	110		146.25
A. pullulans	110		74.75
Total	220		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	1	69.727	.000

Table A5.2.1.18: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Dukaat' flowers treated *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001 and inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N		Mean Rank
5	80		110.50
12	80		110.50
20	60		110.50
Total	220		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	2		

Table A5.2.1.19: ANOVA table for disease severity of freesia var. 'Dukaat' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	32.272	5	6.454	24.638	.000
Intercept	750.491	1	750.491	2864.802	.000
CHEMICAL TREATMENT (C)	31.685	1	31.685	120.948	.000
TEMPERATURE (T)	.000	2	.000	.000	1.000
C * T	.000	2	.000	.000	1.000
Error	56.061	214	.262		
Total	852.722	220			
Corrected Total	88.333	219			

Table A5.2.1.20: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments	N	1	2
A. pullulans 5 [a]	40	1.4810	
A. pullulans 12	40	1.4810	
A. pullulans 20	30	1.4810	
Control 5	40		2.2470
Control 12	40		2.2470
Control 20	30		2.2470
Sig.		1.000	1.000

[a] 5, 12, 20 = 5, 12, and 20°C.

Table A5.2.1.21: ANOVA table for lesion number of freesia var. 'Dukaat' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	30235.519	5	6047.104	6.109	.000
Intercept	1039293.02	1	1039293.02	1049.88	.000
CHEMICAL TREATMENT (C)	5142.154	1	5142.154	5.195	.024
TEMPERATURE (T)	15060.306	2	7530.153	7.607	.001
C * T	8963.572	2	4481.786	4.527	.012
Error	211841.258	214	989.912		
Total	1319097.00	220			
Corrected Total	242076.777	219			

Table A5.2.1.22: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments	N	1	2
A. pullulans 20 [a]	30	62.6333	
Control 20	30	62.8333	
A. pullulans 5	40	63.7000	
Control 5	40	65.2750	
A. pullulans 12	40	67.1250	
Control 12	40		94.6250
Sig.		.599	1.000

[a] 5, 12, 20 = 5, 12, and 20°C.

Table A5.2.1.23: ANOVA table for lesion diameter of freesia var. 'Dukaat' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.831	5	.166	3.107	.010
Intercept	81.274	1	81.274	1518.90 0	.000
CHEMICAL TREATMENT (C)	.195	1	.195	3.651	.058
TEMPERATURE (T)	.423	2	.211	3.950	.021
C * T	.164	2	8.217E-02	1.536	.218
Error	10.381	194	5.351E-02		
Total	94.456	200			
Corrected Total	11.212	199			

Table A5.2.1.24: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with *A. pullulans* at 10^5 c.f.u. mL^{-1} during glasshouse trial 2001. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments		1	2	3
A. pullulans 5 [a]	30	.5450		
A. pullulans 12	40	.5957	.5957	
Control 5	30	.6100	.6100	.6100
Control 20	30		.6877	.6877
A. pullulans 20	30		.6947	.6947
Control 12	40			.7270
Sig.		.287	.116	.061

[a] 5, 12, 20 = 5, 12, and 20°C.

Table A2.1.2.25: ANOVA table for PAL activity of *A. pullulans* treated flowers incubated at 20°C (0 h).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1632.486	1	1632.486	.362	.556
Within Groups	72117.991	16	4507.374		
Total	73750.478	17			

Table A2.1.2.26: ANOVA table for PAL activity of *A. pullulans* treated flowers incubated at 20°C (6 h).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	42389.193	1	42389.193	5.828	.028
Within Groups	116379.487	16	7273.718		
Total	158768.680	17			

Table A2.1.2.27: ANOVA table for PAL activity of *A. pullulans* treated flowers incubated at 20°C (12 h).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.996	1	9.996	.001	.980
Within Groups	255764.233	16	15985.265		
Total	255774.230	17			

Table A2.1.2.28: ANOVA table for PAL activity of *A. pullulans* treated flowers incubated at 20°C (24 h).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4974.345	1	4974.345	3.138	.096
Within Groups	25362.637	16	1585.165		
Total	30336.982	17			

APPENDIX 5.3: METHYL JASMONATE PREHARVEST TREATMENTS

A5.3.1 Effect of preharvest methyl jasmonate treatment (glasshouse trial 2002)

Table A5.3.1.1: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N		Mean Rank
Control	48		109.05
MeJA 1	48		102.74
MeJA 2	48		85.95
MeJA 3	48		88.26
Total	192		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	3	5.921	.116

Table A5.3.1.2: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Cote d'Azur' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N	Mean Rank
5	64	35.11
12	64	127.85
20	64	126.54
Total	192	

Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	118.808	.000

Table A5.3.1.3: ANOVA table for disease severity of freesia var. 'Cote d'Azur' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	198.000	11	18.000	32.452	.000
Intercept	642.769	1	642.769	1158.831	.000
CHEMICAL TREATMENT (C)	4.932	3	1.644	2.964	.034
TEMPERATURE (T)	182.329	2	91.164	164.358	.000
C * T	10.740	6	1.790	3.227	.005
Error	99.841	180	.555		
Total	940.610	192			
Corrected Total	297.841	191			

Table A5.3.1.4: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4
Control 5 [b]	16	.3125			
MeJA 2 5	16	.3313			
MeJA 1 5	16	.4188			
MeJA 3 5	16	.7438			
MeJA 3 20	16		1.9875		
MeJA 2 12	16		2.1125		
MeJA 2 20	16		2.4500	2.4500	
MeJA 3 12	16		2.4688	2.4688	
MeJA 1 20	16		2.5625	2.5625	2.5625
MeJA 1 12	16			2.7312	2.7312
Control 12	16			2.7813	2.7813
Control 20	16				3.0563
Sig.		.137	.050	.271	.087

[a] MeJA 1, 2, 3 = 200, 400, and 600 µM, respectively. Control = 0 µM.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.3.1.5: ANOVA table for lesion number of freesia var. 'Cote d'Azur' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	329616.682	11	29965.153	62.098	.000
Intercept	1016899.63	1	1016899.63	2107.35	.000
CHEMICAL TREATMENT (C)	8862.391	3	2954.130	6.122	.001
TEMPERATURE (T)	314042.167	2	157021.083	325.400	.000
C * T	6712.125	6	1118.688	2.318	.035
Error	86858.688	180	482.548		
Total	1433375.00	192			
Corrected Total	416475.370	191			

Table A5.3.1.6: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	1	2	3	4	5
MeJA 3 5[b]	16	14.1250			
Control 5	16	14.5000			
MeJA 2 5	16	17.0625			
MeJA 1 5	16	17.2500			
MeJA 2 12	16		83.1250		
MeJA 2 20	16		89.6875	89.6875	
MeJA 3 20	16		96.4375	96.4375	96.4375
MeJA 3 12	16		97.5625	97.5625	97.5625
Control 12	16			102.2500	102.2500
MeJA 1 12	16				107.7500
MeJA 1 20	16				112.0000
Control 20	16				121.5625
Sig.		.720	.090	.142	.074	.093

[a] MeJA 1, 2, 3 = 200, 400, and 600 µM, respectively. Control = 0 µM.

[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.3.1.7: ANOVA table for lesion diameter of freesia var. 'Cote d'Azur' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15.077	11	1.371	76.660	.000
Intercept	129.757	1	129.757	7257.45	.000
CHEMICAL TREATMENT (C)	4.403	3	1.468	82.089	.000
TEMPERATURE (T)	8.552	2	4.276	239.163	.000
C * T	2.122	6	.354	19.778	.000
Error	4.076	228	1.788E-02		
Total	148.910	240			
Corrected Total	19.153	239			

Table A5.3.1.8: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Cote d'Azur' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
MeJA 1 5 [b]	20	.4550				
MeJA 3 5	20	.4590				
MeJA 2 5	20	.4715				
Control 5	20	.4880				
MeJA 3 20	20		.6385			
MeJA 1 20	20			.7505		
MeJA 1 12	20			.7705		
MeJA 3 12	20			.7730		
MeJA 2 12	20			.7935		
MeJA 2 20	20			.8140		
Control 12	20				1.1545	
Control 20	20					1.2555
Sig.		.485	1.000	.186	1.000	1.000

[a] MeJA 1, 2, 3 = 200, 400, and 600 µM, respectively. Control = 0 µM.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.3.1.9: Parameters estimated for the linear model ($y = y_0 + ax + bx^2$) used to describe the effects of MeJA concentration and incubation temperature on disease severity, lesion number and lesion diameter on var. 'Cote d'Azur' freesia petals.

Temperature (°C)	Estimated parameters			Coefficient (R^2)
	y_0	a	b	
a. Disease severity				
5	0.34	-0.0006	0	0.77
12	2.88	-0.0026	0	0.61
20	3.06	-0.0017	0	0.95
b. Lesion number				
5	14.95	0.017	0	0.99
12	105.55	-0.052	0.0001	0.26
20	124	-0.11	0.0001	0.88
c. Lesion diameter				
5	-0.49	-0.0001	0	0.70
12	1.13	-0.0019	0	0.91
20	1.22	-0.0022	0	0.86

Table A5.3.1.9: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Dukaat' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Treatments	N		Mean Rank
Control	81		188.06
MeJA 1	81		147.90
MeJA 2	81		150.30
MeJA 3	81		163.74
Total	324		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	4	9.767	.021

Table A5.3.1.10: Non-parametric test (Kruskal-Wallis) for disease severity of freesia var. 'Dukaat' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1}.

Temperature	N		Mean Rank
5	108		100.16
12	108		216.78
20	108		170.56
Total	324		
Variable	df	Chi-square	Asymp. Sig.
Disease severity	2	88.360	.000

Table A5.3.1.11: ANOVA table for disease severity of freesia var. 'Dukaat' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	39.463	11	3.588	12.908	.000
Intercept	156.111	1	156.111	561.683	.000
CHEMICAL TREATMENT (C)	3.902	3	1.301	4.680	.003
TEMPERATURE (T)	31.835	2	15.917	57.270	.000
C * T	3.726	6	.621	2.235	.040
Error	86.716	312	.278		
Total	282.290	324			
Corrected Total	126.179	323			

Table A5.3.1.12: Disease severity means separated according to Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]		1	2	3	4	5
MeJA 2 5 [b]	27	.2148				
MeJA 1 5	27	.2815				
Control 5	27	.2852				
MeJA 3 5	27	.3778	.3778			
MeJA 1 20	27	.4889	.4889	.4889		
MeJA 2 20	27		.6148	.6148		
MeJA 3 20	27			.7481	.7481	
MeJA 3 12	27				.9333	.9333
MeJA 2 12	27				.9593	.9593
MeJA 1 12	27					1.0852
Control 20	27					1.1037
Control 12	27					1.2370
Sig.		.089	.119	.087	.166	.058

[a] MeJA 1, 2, 3 = 200, 400, and 600 µM, respectively. Control = 0 µM.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.3.1.13: ANOVA table for lesion number of freesia var. 'Dukaat' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	41170.182	11	3742.744	10.790	.000
Intercept	235818.151	1	235818.151	679.838	.000
CHEMICAL TREATMENT (C)	6656.404	3	2218.801	6.397	.000
TEMPERATURE (T)	27411.062	2	13705.531	39.512	.000
C * T	7102.716	6	1183.786	3.413	.003
Error	108224.667	312	346.874		
Total	385213.000	324			
Corrected Total	149394.849	323			

Table A5.3.1.14: Lesion number means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	1	2	3	4	5	6	7
MeJA 2 5 [b]	27	11.8148					
Control 5	27	13.7407	13.7407				
MeJA 1 5	27	15.0000	15.0000				
MeJA 1 20	27	16.2222	16.2222	16.2222			
MeJA 3 5	27	21.4815	21.4815	21.4815			
MeJA 2 20	27		24.1111	24.1111	24.1111		
MeJA 3 20	27			26.4444	26.4444	26.4444	
MeJA 2 12	27				33.1481	33.1481	33.1481
MeJA 3 12	27					36.0000	36.0000
MeJA 1 12	27						37.5556
Control 20	27						42.8148
Control 12	27						45.4074
Sig.		.090	.068	.065	.092	.074	.081	.090

[a] MeJA 1, 2, 3 = 200, 400, and 600 µM, respectively. Control = 0 µM.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.3.1.15: ANOVA table for lesion diameter of freesia var. 'Dukaat' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.345	11	.213	25.875	.000
Intercept	48.884	1	48.884	5932.94	.000
CHEMICAL TREATMENT (C)	.657	3	.219	26.568	.000
TEMPERATURE (T)	.836	2	.418	50.727	.000
C * T	.852	6	.142	17.243	.000
Error	.890	108	8.239E-03		
Total	52.119	120			
Corrected Total	3.235	119			

Table A5.3.1.16: Lesion diameter means separated using Duncan's multiple range test at P = 0.05. Freesia var. 'Dukaat' flowers treated with MeJA at three rates during MeJA glasshouse trial 2002. Flowers were inoculated with 10^4 *B. cinerea* conidia mL^{-1} and incubated at 5, 12, and 20°C. Numbers on the same column are not significantly different at P = 0.05

Treatments [a]	1	2	3	4
MeJA 3 5 [b]	10	.4940		
MeJA 2 20	10	.5010		
MeJA 1 5	10	.5080		
MeJA 2 5	10	.5180		
MeJA 1 20	10	.5410		
Control 5	10	.5650		
MeJA 1 12	10		.6530	
MeJA 3 12	10		.7080	.7080
MeJA 3 20	10		.7100	.7100
Control 12	10		.7300	.7300
MeJA 2 12	10			.7500
Control 20	10			.9810
Sig.		.130	.086	.353	1.000

[a] MeJA 1, 2, 3 = 200, 400, and 600 µM, respectively. Control = 0 µM.
[b] 5, 12, 20 = 5, 12, and 20°C.

Table A5.3.1.17: Parameters estimated for the linear model ($y = y_0 + ax + bx^2$) used to describe the effects of MeJA concentration and incubation temperature on disease severity, lesion number and lesion diameter on freesia var. 'Dukaat' petals.

Temperature (°C)	Estimated parameters			Coefficient (R^2)
	y_0	a	b	
a. Disease severity				
5	0.32	0.0006	0	0.60
12	1.2	-0.0005	0	1
20	1.07	-0.0034	0	0.91
b. Lesion number				
5	14.85	-0.023	0.0001	0.75
12	45.3	-0.054	0.0001	0.98
20	40.95	-0.13	0.002	0.78
c. Lesion diameter				
5	0.57	-0.0002	0	0.82
12	0.71	-0.0001	0	0.86
20	0.97	-0.0029	0	0.99